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GENES and POLITICS

The science of Genetics arose to study the transmission of physical characteristics from parents to their offspring. When closely studied, much variation exists for virtually any characteristic, say, in size or color, among the members of all species, be they flies, dogs, or ourselves, the members of the *Homo sapiens* species. The origin of this variability long fascinated the scientific world, which already in the 19th century asked how much of this variation is due to environmental causes (nurture) as opposed to innate hereditary factors (nature) that pass unchanged from parents to offspring. That such innate heredity exists could never be realistically debated. One need just look at how characteristics in the shape of the face pass through families. Ascribing, say, the uniqueness of the Windsor face to nurture as opposed to nature goes beyond the realm of credibility.

Genes as the Source of Hereditary Variation Both within and between Species

The key conceptual breakthrough in understanding the nature component of variation came in the mid 1860s from the experiments of the Austrian monk and plant breeder, Gregor Mendel (1822–1884). In his monastery gardens he created, by self-breeding, strains of peas that bred true for a given character like pea color or pod shape. Then he crossed his inbred strains with each other and observed how the various traits assorted in the progeny pea plants. In his seminal scientific paper, published in 1865, Mendel showed that the origin of this hereditary variability lay in differences in discrete factors (genes) that pass unchanged from one plant generation to another.
Most importantly, he showed that each pea has two sets of these factors, one coming from the male parent, the other from the female. Some of those factors are expressed when present in only one copy (dominant genes), whereas others become expressed only when two copies, one from each parent, are present (recessive genes). Mendel’s results later were used by the Danish botanist, Wilhelm Johannsen (1857–1927), to make the important distinction between the physical appearance of an individual (its phenotype) and its genetic composition (genotype). Mere examination of a plant’s physical appearance need not reveal its genetic composition. Recessive genes present in only one copy can be identified only by further genetic crosses. Mendel further made the equally important observation that genes do not necessarily stay together when the male and female sex cells are formed. Instead, they often independently assort from each other, giving rise to progeny with sets of features very different from those of either parent.

Mendel’s work, done before the behavior of chromosomes during cell division was understood, almost had to lay unappreciated until the turn of the century, when three plant breeders working on the European continent, Correns, De Vries, and Tschermak, independently rediscovered the basic rules for hereditary transmission, which today we call Mendel’s Laws. It was not until 1890 that the sex cells were found to possess only half the number of chromosomes present in adult cells. Fertilization through combining the haploid $N$ number of chromosomes of the sperm with the haploid $N$ number of the egg restores the $2N$ diploid chromosome number of adult plants and animals. Except for those special chromosomes that determine sex, adult cells contain two copies of each distinct chromosome, each of which is exactly duplicated prior to the cell division. With the basic facts of chromosome behavior so established for both ordinary cell division (mitosis) and sex cell formation (meiosis), the rediscovered laws of Mendel were given a chromosomal basis by the American, Walter Sutton. Perceptively, he noted in 1903 that the segregation patterns of Mendel’s genes exactly parallel the behavior of chromosomes during the meiotic cell divisions that produce the male and female sex cells (The Chromosomal Theory of Heredity). During the next several decades, an ever-increasing number of genes were found to have precise locations along specific chromosomes. In essence, each chromosome came to be seen as a linear collection of genes running between its two ends.

Genes first were of interest because they were the source of the variability between the members of a species, but they soon began to be appreciated more properly as the source of information that gives an organism its unique form and function. Its collection of genes (its genome) is what gives each organism its own unique developmental pathway. A dog is a dog, a bacterium a bacterium, etc., because of the information carried by their respective genomes. Gene duplication prior to cell division thus must be based on a very accurate copying process. Otherwise, there would be no constancy of genetic information and of the development processes they make possible. Correspondingly, genetic variation arises when genes are not accurately copied (mutated) and give rise to changed (mutant) genes.

**Hereditary Variability Generated by Changes in Genes (Mutation)**

**Underlies Evolution by Natural Selection**

As soon as the first spontaneous gene mutations became known, they were perceived as the obvious source of the new genetic variants necessary for Darwinian Evolution by "survival of the fittest." Many more dysfunctional than more functional genes, however, resulted from random
mistakes in the gene copying process. Thus, the rate at which the gene-copying process makes mistakes is likely also to be under strong evolutionary pressure. If too many spontaneous mutations occur, none of the mutant-gene-bearing organisms are likely to develop and produce viable offspring. Correspondingly, too low a mutation rate will not generate sufficient gene variants to allow species to compete effectively with those species evolving faster because of their more frequent generation of biologically fitter offspring.

Eugenic Solutions for Human Betterment

The coming together of Darwinian and Mendelian thinking immediately raised the question of the applicability of the new science of genetics to human life. To what extent was human success due to the presence in their recipients of good genes that led to useful biological traits like good health, social dependability, and high intelligence? Correspondingly, how many individuals at the bottom of the human success totem pole were there because they possessed gene variants perhaps useful for earlier stages in human evolution but now inadequate for modern urbanized life? Social Darwinian reasoning viewed the sociocultural advances marking humans' ascent from the apes as the result of continual intergroup and interpersonal strife, with such competitive situations invariably selecting for the survival of humans of ever-increasing capabilities. Social Darwinism came naturally to the monied products of the industrial revolution, a most prominent one being the talented statistician, Francis Galton (1822–1911). Early in his career, he wrote the 1869 treatise *Hereditary Genius*, later coining the term "eugenics" (from the Greek meaning well-born) for studies that would bring about improvements of the human race through the careful selection of parents.

Clever though he was, and able to take comfort that he was Charles Darwin's (1809–1882) cousin, Galton's eugenic prescriptions offered no basic improvement on the long-attempted practice of seeing that offspring from families of attainment married into families of similar high function. In this way, supposed good germ plasm would not be diluted by inputs of putative bad heredity. But whether Galton was promoting reality, as opposed to an unjustified prejudice against the vulgarity of the lower classes, had no way of being even half-tested before the arrival of Mendelian analysis. So the eugenics movement naturally became galvanized by the new laws of Mendelian heredity. But immediately, their hopes had to be tempered by the fact that human genetics never would have the power of other forms of genetics where genetic crosses could be made as well as observed. For better or worse, the eugenicists' main research tool had to be hopefully well-collected, multi-generational pedigrees of physical and mental traits that passed through families from one generation to the next. Toward that end, Galton, then already 84, co-authored in 1906 the book *Noteworthy Families*, an index to kinships in near degrees between persons whose achievements are honorable and have been publicly recorded.

Initially, there were hopes that simple Mendelian ratios would characterize the inheritance of a broad-ranging group of human traits. But in addition to the limitations brought about through the inability to confirm genetic hypotheses through genetic crosses, many of the studied traits appeared in too few families for appropriate statistical analysis. Particularly difficult to analyze were progeny traits not present in either parent. Conceivably, individuals had inherited one copy of the same recessive gene from each parent. Such tentative conclusions became more convincing when the respective traits, like albinism, were found more often in highly inbred, isolated populations where marriages of cousins were frequent.
Easier to assign as bona fide genetic determinants were dominant-acting genes that need be inherited from only one parent for their presence to be felt. Once Mendelian thinking had appeared, the inheritance mode of Huntington's Disease, the terrible neurological disease that leads to movement and cognition disorders, was quickly ascertained as a dominant gene disorder. Similar clear genetic attributions could be assigned to traits, such as red-green color blindness and hemophilia, which preferentially appear in males but which are never passed on to their own male offspring. This is the behavior of a trait caused by a gene present on the X sex chromosome, two copies of which are present in females but only one in males, whose sexuality is determined by the Y chromosome.

Important as these diseases of the body were to the individuals and families of those so afflicted, the main focus of early 20th century eugenicists soon moved to potential genetic causations for disabilities of the mind, embracing a wide spectrum of manifestations from insanity through mental defectiveness, alcoholism, and criminality, to immorality. With poorhouses, orphanages, jails, and mental asylums all too long prominent features of the most civilized societies, eugenicists with virtually religious fervor wanted to prevent more such personal and societal tragedies in the future. They also desired to reduce the financial burdens incumbent on civilized society’s need to take care of individuals unable to look after themselves. But in their evangelical assertions that genetic causations lay behind a wide variety of human mental dysfunctions, the early eugenically focused geneticists practiced sloppy, if not downright bad, science and increasingly worried their more rigorous geneticist colleagues.

American Eugenics: Sloppy Genetics for the Legitimation of Class Stratification

The most notable American eugenicist, whose conclusions went far beyond his facts, was Charles B. Davenport (1866-1944), who parlayed his position as Director of the Genetics Laboratory at Cold Spring Harbor, New York, to establish in 1910 a Eugenics Record Office using monies provided by the widow of the railway magnate E.H. Harriman. In his 1911 book *Heredity in Relation to Eugenics*, pedigrees were illustrated for a wide-ranging group of putative hereditary afflictions ranging from bona fide genetic diseases, such as Huntington’s disease and hemophilia, to behavioral traits of much less certain hereditary attribution, such as artistic ability and mechanical ability with reference to shipbuilding. With so little then known about the functioning of the human brain, Davenport’s early rush to associate highly specific accomplishments of the human brain to specific genetic determinants could not automatically be dismissed as nonsense. In today’s intellectual climate, however, a predilection for genes that predispose individuals to city life as opposed to rural life would not be the way to an academic career. But, even his fellow early eugenicists must have regarded, as more wartime patriotism than science, his 1917 claim that a dominant gene for thalassophilia predisposed its recipients to careers as naval captains.

In addition to its family pedigree assembly and archival roles, the Cold Spring Harbor Eugenics Record Office frequently counseled individuals with family backgrounds of genetic diseases, particularly when they were considering marriage to blood relatives. Many such seekers of help must have been misled by advice that never should have been given, considering that era’s limited power for meaningful genetic analysis. Worries about insanity were a major concern, where manic-depressive disease was seen to move through some families as if
it were a dominant trait. In contrast, schizophrenia had more aspects of a recessive disease. Yet, even with today's much more powerful human genetics methodologies, we still do not know the relative contribution of dominant versus recessive genes to these two major psychoses or any other form of mental disease.

The Eugenics Record Office's pre-World War II message was that insanity usually expressed itself only when genes predisposing it were inherited from both parents. If this were so, siblings of individuals displaying mental instability were at risk of being carriers of insanity-provoking genes. Since recessive genes for insanity would be silently passing through many families, marriage to any individual with mentally disturbed siblings was not prudent. Even more certainly, marriage should be avoided between individuals having severe mental illness in both parents. In those days, when no effective medicines existed for any form of psychiatric illness, most families bearing mental disease not surprisingly kept this knowledge as secret as possible. There must have been many couples, perhaps over-worried about producing mentally disturbed offspring, who chose not to have children.

The eugenicists predictably were concerned about mentally unstable individuals marrying those with similar disturbances. Also of obsessive concern to them were individuals with feeble-mindedness, where Davenport believed that recessive genes were also involved. With his certainty that all children of two feeble-minded parents would be defective, he wrote of the "Folly, yes the crime, of letting two such persons marry." In his mind, the inhabitants of rural poorhouses were there largely because of their feeble-mindedness, and he considered one of our nation's worst dangers to be the constant generation of feeble-minded individuals by the unrestrained lusts of parents of similar conditions.

It was to stop such further contaminations of the American germ plasm that Davenport, already as early as 1911, saw the need for state control of the propagation of the mentally unstable or defective. Initially, he did not favor adoption of state laws allowing for their compulsory sterilization, an idea then considered wise and humane by much of that era's socially progressive elite. Clearly somewhat sexually repressed (obsessed?), he feared that with pregnancy no longer a worry, the sexual urges of the sterilized, mentally unstable impaired might cause more harm to society than even the procreation of more of their kind. Instead, he wanted mentally impaired women to be effectively segregated (imprisoned?) from the impaired of the opposite sex until after they passed the age of procreation. This prescription, however, was totally unrealistic and the American eugenics movement as a whole enthusiastically promoted the compulsory sterilization legislation that spread to 30 states by the start of World War II.

If the eugenics movement had focused its attention predominately on genetic afflictions that truly disabled its recipients, we might now be able to look back at it as a mixture of sloppy science and well-intentioned but kooky naiveté. Photos of the Eugenics Booths of 1920s State Farm Fairs are virtually laughable. In them can be seen "fitter families* displayed near the pens at which prize cattle were shown. The thought that sights of their earnest faces would lead to preferential procreation of more of the same now stretches our credulity. In contrast, the words and actions of Harry P. Laughlin, Davenport's close associate and Superintendent of the Eugenics Record Office, today can only make our minds flinch.

Pleased that his ancestors were traceable to the American Revolution, Laughlin shared Davenport's belief that the strengths and weaknesses of national and religious groups were rooted in genetic as well as in cultural origins. While, at least in public, Davenport wrote that no individual should be refused admission to the United States on the basis of religious group or national origin, Laughlin stated as scientific fact before appropriate Congressional bodies that
the new Americans from Eastern and Southern Europe were marked by unacceptable amounts of insanity, mental deficiency, and criminality. Although he lacked any solid evidence, he nonetheless promoted the belief that the newest immigrants to our shores were much more likely to be found in prisons and insane asylums than were the descendants of earlier waves of English, Irish, German, and Scandinavian settlers. Even though the then-current postwar hysteria against unrestrained immigration by itself might have led to the 1924 legislation, there is no doubt that Laughlin's testimony tilted the composition of the future immigrants to Northern Europeans.

With legislation in place, Davenport no longer had to fear that "the population of the United States will on account of the great influence of blood (genes) from South-Eastern Europe rapidly become darker in pigmentation, smaller in stature, more mercurial, more attached to music and art, given to crimes of larceny, kidnapping, assault, murder, rape, and sex immorality and less given to burglary, drunkenness, and vagrancy than were the original English settlers." Through propagating such racial and religious prejudices as scientific truths, the American eugenics movement was, in effect, an important ally of the ruling classes, many of whose privileges inevitably came through treating those less fortunate as inherently unequal.

Using the First IQ Tests to Justify Racial Discrimination within the United States

The emergence of intelligence measuring reinforced the belief of America's prosperous people that their wealth reflected their respective family's innate intellectual superiority. The French psychologist, Alfred Binet (1857-1911), was the first person to try to systematically measure intelligence, responding to a 1904 request from the French government to detect mentally deficient children. The resulting Binet-Simon tests crossed the Atlantic by 1908, being first deployed in the United States by Henry Goddard in New Jersey at a training school for feeble-minded boys and girls. Soon afterward, he went on to test 2000 children with a broad range of mental abilities. Initially, there was considerable public opposition to the testing of "normal" individuals because of the test's first use on the feeble-minded. Within only a few years, however, revised Binet-Simon tests, more appropriate for precocious children, were prepared by Lewis Ternan (1877-1956) at Stanford University. These so-called IQ (Intelligence Quotient) tests were soon employed during World War I on hundreds of thousands of army draftees. Their main function was not to weed out mental defectives, but to assign recruits to appropriate army roles. Those administering the tests, led by the noted psychologist Robert M. Yerkes (1876-1956), claimed they were seeing native intelligence independent of the recruit's environmental history. Yet, clearly, many of the questions or arithmetic problems would be more easily answered by those with extensive schooling and possessing a broad vocabulary. Not surprisingly the non-English-speaking recruits just off the immigration boats tested badly, allowing a test leader to privately confide to Davenport, "we are well on the right track in our contention that the germ plasm (now) coming into the country does not carry the possibilities of that arriving earlier." Such "objective test data" further convinced the eugenicist world that not only was mental deficiency genetically determined, but so was general intelligence.

Although black men from urban areas tested higher than white southern rural men, their IQ scores were significantly lower than their white equivalents from the same communities. Given today's realization that intelligence measurements virtually by necessity have cultural biases, the comparative data assembled from the army recruits had little real meaning. In many ways, it was
like comparing oranges with apples. Nonetheless, the data summarized in *Psychological
Examining in the United States Army* were used to justify the discriminatory segregation laws
that effectively made America’s black population second-class citizens. Genetic inequalities
across so-called race boundaries were taken for granted, and 29 states maintained laws against
black-white intermarriages often using the argument that the superior white germ stock would be
diluted with inferior genes.

Although eugenics had its origin in England, it never affected the national consciousness
there as it did in the United States. With social class stratification so long a characteristic feature
of British life, the ruling classes had no need of further justification for their privileged existence.
To a lesser but real extent, social inequalities also were taken-for-granted features of most
European countries, many of which still had royal families and their attendant aristocracy.
Enthusiastic prewar eugenics movements nonetheless sprang up all over the continent,
extending even to Southern America and Japan in the 1920s. Everywhere, the chief adherents
were the professional middle class, naively proselytized into believing that genetic thinking
could soon lead to human beings with heightened hereditary capabilities. Although the
continent’s eugenicists frequently used the now unacceptable term "race hygiene" for their
movement, their ways for the betterment of human hereditarily for the most part in no way infringed
upon preexisting human liberties. Offered as the future panacea was the standard package of
marriage between genetically healthy individuals, with correspondingly strong disapproval of
marriage for individuals bearing obviously bad genes like those leading to Huntington’s Disease.
Only in two European countries, Germany and Sweden, was legislation enacted for obligatory
sterilization of individuals thought to be the bearers of disabling genes.

Nazi Eugenics (Race Hygiene): A Murderous Ménage à Trois of
Bad Genetics, Racial Anthropology, and Psychiatry at the Beckon
of Hitler and Himmler

Although it was the Hitler-led Nazi government that quickly passed the 1933 Eugenic
Sterilization Law, the broadly based German eugenics movement of the 1920s laid the ground
work. Then it was embraced by a spectrum of political thought, much of it totally respectable by
the ethical standards of those days. The Germany of that time was a nation undergoing a great
moral crisis brought on by its humiliating defeat in the World War. Its four awful years of trench
warfare had killed a significant fraction of its better younger men and left it vulnerable to the
hyperinflation that wiped out much of the savings of its professional middle class. Unlike
England or France, Germany as a world power had only a fleeting existence, and the German
people then saw the need to somehow reinvigorate themselves. The eugenicists’ vision that
human beings’ futures lie in their genes struck a receptive chord in the immediate postwar
German psyche. Even in the postwar chaos of its Weimar government, human genetics gained
strong governmental support. Genetics quickly became a high-quality science in Germany, with
Berlin becoming one of the world’s leading centers for genetics. Study of supposed genetic
differences between the so-called races was vigorously promoted, with it being accepted as
fact that the commercial colonization of the world by major countries in Europe and the United
States reflected the inherent superiority of the Nordic people’s genes for intelligence and
strength of moral purpose. Anthropological-based research had strong genetic components,
with genes being perceived as the crucial element determining human behavior.
In total contrast, genetic explanations for human successes were not favorably received in the Soviet Union, whose communist doctrines emphasized social, as opposed to genetic, causation for the currently existing inequalities between humans. Already by the mid 1930s, eugenic thinking had become strongly inimical to Russian Communist policymakers, who increasingly favored the Lamarckian explanations (inheritance of acquired characteristics) of Trofim Lysenko (1898–1982), its homegrown agriculturist, over the foreign-originating Morgan-Mendelian analysis of heredity. Those pursuing genes within the Soviet Union soon were putting not only their careers, but also their lives at risk. The great American geneticist, Herman J. Muller (1890–1967), whose left-wing views led him to leave Texas and come to Russia in 1933, effectively ended his Soviet career when, in 1936, he compared Lamarckian thinking to alchemy, astrology, and shamism.

Seeking backing for the putative superiority of the Caucasian race, Adolf Hitler (1889–1945), while imprisoned in 1924 for the failed Munich putsch, read Menschliche Erblichkeitslehre and Rassenhygiene (The Principles of Human Heredity and Race Hygiene), a leading German genetic text of the time coauthored by E. Baur, Eugen Fischer, and Fritz Lenz. Enveloped by an uncritical eugenic perspective, it strongly reinforced Hitler’s view of Germans as the master race that justifiably should rule the world. If, however, the Germans were indeed the master race, the Nazis had to explain their nation’s humiliating defeat in the Great War and its subsequent devastating hyperinflation.

A perfidious scapegoat was needed, and here Hitler drew upon the long-existing, anti-Semitic feelings of many German people. Long segregated in rural enclaves dating back to the Middle Ages, Jews became effectively part of Germany’s commercial and professional life only by the middle of the 19th century. Gravitating especially to the professions where their talents could more easily prevail over still-existing prejudices, Jewish importance in German commerce and professional life soon became disproportionate to their numbers, creating jealousies that inevitably fanned preexisting anti-Semitism. Clearly, much of this Jewish success reflected its religion and its respect for the intellect as opposed to oft revelatory-based opinions of their Christian equivalency. Their anti-Semitic opponents, however, saw the Jews’ upward trajectory as manifestation of inherent immorality that let them take unfair advantage of the more honest Christian Germans.

Until the arrival of Mendelian thinking, German anti-Semites never consistently decided whether their enemy was the Jews themselves or their religion. If their failure to acknowledge Christ was the problem, Jews who converted posed no further threat to Christian civilization. But if their reputed unscrupulous behavior and sexual licentiousness reflected innate hereditary qualities, their presence within Christian societies threatened their country’s moral resolve, if not its very existence. Assertions by eugenicists that gene differences lay behind human behavioral differences were thus made to order for Nazi needs. From the 1933 start of their absolute rule, the Nazi propaganda machine ruthlessly portrayed Jews and Communists as the two main villains blocking the ultimate triumph of National Socialism. No words were vile enough to express their hatred for the genes that supposedly let Germany’s one million Jews steal for themselves the monies and jobs of the honest Germans, or their horror of the Communists who wanted to redistribute monies from those who worked hard to those not able or willing to take care of themselves. Treated with equal contempt by the Nazis, but of less importance because of their much smaller numbers (30,000), were the Gypsies. Because of their wandering, supposed sexually unrepressed life styles, and their lack of respect for property, the German Gypsies were regarded by Nazi anthropologists as descendants of peoples of primitive etiological origin who had mated repeatedly with the German criminal, asocial subproletariat. So considered, the further breeding of this mixed blood people must be stopped.
Sterilization of the Mentally Unfit as a Prelude to More Broadly Based Wartime Genocide

Gypsies, however, were not specifically targeted under the 1933 Eugenics Law that mandated compulsory sterilization for schizophrenia, manic-depressive psychoses, hereditary epilepsy, Huntington’s Chorea (Disease), hereditary blindness, hereditary deafness, severe physical deformity, and severe alcoholism. Tribunals of Hereditary Health, consisting of a judge, a government medical officer, and an "independent" physician, made the resulting decisions where the individuals concerned often knew they were at risk only when called before its members. With appeals extremely difficult, these psychiatrist-led verdicts between 1934 and 1939 led to some 400,000 compulsory sterilizations, many to noninstitutionalized persons. These reputedly hereditary-damned individuals were further subjected to a subsequent 1934 law forbidding persons with serious mental disturbances from marrying. A year later, legislation specifically affecting Jewish marriages came through the 1935 "Nuremberg Decrees" for the protection of German blood and health. They forbade not only marriages, but also sexual intercourse between the so-called German and Jewish races.

Concomitant with these eugenic actions was the assembly of vast record collections documenting individual hereditary-biological characteristics. To the Reich Kinship Bureau were referred decisions as to the origin of individuals with potential partial Jewish blood. Many such anthropological "expert conclusions" were made using only photos of the putative fathers. However, with the census of 17 May 1939 providing supposed "confidential" information of any Jewish grandparent, the Nazis, as the war started, felt they had a firm handle on the Jewish blood within their midst. So encouraged, later that year Professor Eugen Fischer, this time responding to coal barons of the Ruhr, wrote, "When a people wants somehow or other to preserve its own nature, it must reject alien racial elements and when they have already insinuated themselves, it must suppress and then eliminate them. The Jew is such an alien..."

With the war on, the German government, seeing no reason to waste scarce resources to keep what they considered genetically inferior peoples alive, proceeded to what it termed an "euthanasia policy of mercy killing." In a one-sentence letter postdated 1 September 1939, Hitler himself wrote, "Reichleiter Bouhler and Dr. Brandt are entrusted with responsibility of extending the rights of specifically designated physicians such that patients who are judged incurable after the most thorough review of their condition which is possible can be granted mercy killing." So authorized, 3000 mental patients in occupied Poland were summarily shot by storm troopers. In the Reich itself, where German citizens were involved, somewhat more formal procedures were used. Questionnaires were distributed to the mental hospitals, where they were completed in their capacity as experts by nine Professors of Psychiatry assisted by 39 other medical doctors. For their labors, they were paid 5 pfennigs (the cost of a cigarette) per questionnaire when they processed more than 3500 per month, but up to 10 pfennigs when fewer than 500 questionnaires per month were processed. The patients so selected for "euthanasia" had their respective questionnaires marked with a cross. Subsequently, carbon monoxide supplied by I.G. Farben was used for the elimination process. Before the killings stopped in the fall of 1941, some 94,000 mental patients had been killed. Subsequently, covert "euthanasia" by starvation, drugs, and failure to treat infectious diseases led to only 15% (40,000 persons) of Germany’s prewar mental hospital population remaining alive at the war’s end.

The primary reason for the supposed stopping of mental patient "mercy killings" was the need to transfer the personnel trained in killing by gas to the concentration camps, primarily in Poland (e.g., Auschwitz), to which most German Jews and gypsies had already been deported. With the decision already taken to invade the Soviet Union, a conference was held in March 1941 in
Frankfurt at the Institute for the Investigation of the Jewish Question. At this conference, Dr. Gross, the head of the Race Policy Institute of the Nazi party, stated, "The definitive solution must involve the removal of Jews from Europe and he demands sterilization of quarter Jews." In an October letter to Himmler, Oberdiensleiter Brack of the Fuhrer’s chancellery wrote that there are no objections to doing away (gassing) Jews who are unfit for concentration camp work. Less than a month later, Rosenberg, theoretician and minister of the occupied eastern territories, before representatives of the German press, announced the Final Solution of the Jewish Question, revealing plans, still to be kept secret, for the eventual mass murder of all European Jews, including the six million then living in the Soviet Union. The gas chambers so used were in no way restricted to Jews and Gypsies, with Soviet prisoners of war being victims of the first uses of Zyklon B (hydrocyanic acid) at Auschwitz. Anxious to give their racial extermination policies "scientific" justification, Himmler later in 1943 specified in a decree that only physicians trained in anthropology should carry out selection for killing and supervise the killings themselves in extermination camps. Some quarter Jews were to be spared, but not those with Jewish facial features who should be treated as half Jews. By the war’s end, five to six million European Jews were so killed, the majority by the gassing procedures that the Nazis’ co-opted human geneticists, psychiatrists, and anthropologists thought appropriate for individuals bearing genes inimical to the best interests of the German people.

With the liberation first of Poland and then Germany, the full horror of the racially based genocide policies of National Socialism quickly became known, generating even further disgust for the pseudoscientific theories of race superiority and purity that underpinned Nazi ideology. Anyone subsequently calling himself a eugenicist put his reputation as a decent moral human at risk. In fact, before the war even started, eugenics in the United States already was being perceived more as a social than a scientific movement. Already in 1930, the leaders of the Carnegie Institute of Washington had been told that its Cold Spring Harbor Eugenics Record Station practiced sloppy if not dishonest science. But with its founder Charles Davenport nearing retirement, it was allowed to expire more slowly than in retrospect it should have. Its doors closed only when Miloslav Demerec became director of the Department of Genetics in 1942. There thus was the embarrassment of Harry Laughlin’s receipt in 1936 of an honorary degree from the University of Heidelberg in recognition of his contributions to racial hygiene. Undoubtedly pleased that eugenics, then fading in the United States, was becoming even more ascendant in Germany, Laughlin went to New York to receive his diploma from the German Diplomatic Counsel.

**Eugenics, a Dirty Word, as the Search for the Chemical Nature of the Gene Begins**

By the time I first came to Cold Spring Harbor for the summer of 1948, accompanying my Ph.D. supervisor Salvador Luria, then a professor at Indiana University, the Eugenics Record Office had been virtually expunged from its consciousness. Only in the library was its ugly past revealed through the German journals of the 1920s and 1930s on Human Genetics and Race Hygiene. No one that summer showed any interest in human genetics as a science or toward the general question of how much of human behavior reflects nature as opposed to nurture. Instead, genetic research there focused on the fundamental nature of genes and their functioning. It was not that human genetic diseases had suddenly become unimportant to its director, Miloslav Demerec. But there was general agreement both by the year-round staff and the many summer visitors that until the chemical identity of the gene was elucidated and the general pathways by
which it controlled cell structure and functioning were known, it was premature to even speculate how genes contributed to human development and behavior.

Then, much sooner than anyone expected, the gene was revealed in 1953 to be DNA. The genetic code was established by 1966, and gene expression was seen to be controlled by DNA-binding regulatory proteins between 1967 and 1969. Genetics, happily then, had no reasons to intersect politics, except in Russia, where the absurdity of its Lamarckian philosophy became painfully more clear to its intelligentsia with every new major advance in molecular genetics. These major genetic breakthroughs were largely accomplished using the simple genetic systems provided by bacteria and their viruses that go under the name phages. By 1969, phage had become so well understood genetically that it became possible to create specific phage strains cleverly engineered to carry specific bacterial genes from one bacterial strain to another. Yet, seeming more ashamed than pleased with their neat science, James Shapiro and Jonathan Beckwith of Harvard Medical School held with much fanfare a press conference to announce that their new way to isolate specific genes was on the pathway to eugenically motivated genetic engineering of human beings. Knowing of the left-wing views of their "Science for the People" group, I, like most of my colleagues in the Boston region, saw their self-denunciations as manifestations of unrepentant leftist fears that further genetic research would render inviable the Communist dogma that assigned all social inequalities to capitalistic selfishness. Shapiro then moved (temporarily) to Cuba to regain his ideological purity.

Although the phage transductional system developed by Shapiro and Beckwith proved not to be a forerunner for eventual human genetic engineering, this was not true for the much more powerful and general "recombinant DNA" methodologies that Herbert Boyer and Stanley Cohen developed 4 years later, in 1973, just 20 years after the discovery of the double helix. Their new procedures allowed the isolation (cloning) of specific genes, through their insertion into tiny chromosomes (plasmids) that could be moved from one cell to another. At roughly the same time, unexpectedly powerful new ways to determine the exact sequences of the four letters (A, G, T, and C) of genetic messages were worked out by Fred Sanger in Cambridge, England, and by Walter Gilbert and Alan Maxam at Harvard. Together, using these two new techniques, the exact structure of any gene could eventually be determined, given the appropriate facilities and resources.

The resulting recombinant DNA era, however, despite all the promises it held for major scientific advances, did not immediately take off. It initially stalled because of fears that among the many new forms of DNA created in the laboratory would be some that would pose unacceptable dangers to life as it now exists. In particular was the fear that highly pathogenic new forms of viruses and bacteria would be created. To give time to assess such potential dangers scientifically, a scientist-initiated moratorium on recombinant DNA research was declared in 1975. Effectively, it blocked virtually all recombinant DNA research for the next 2 years, and research concerned with cancer, where worries were expressed that a cancer gene might become bacterially transmitted, was held up for 2 more years.

During the moratorium, governmental committees were set up in the United States and in various European countries to assess the potential dangers from recombinant DNA experimentation in relation to its potential benefits for biology, medicine, and agriculture. No plausible scientific reasons for stopping such research emerged, and such committees, often containing public as well as scientific representatives, invariably concluded that in the absence of any quantifiable potential dangers, it would be irresponsible not to move ahead with experiments that could dramatically change the nature of biology. In retrospect, these decisions to move ahead were always the correct ones. For example, cancer research and our knowledge...
of the genetic basis of the immune system, would effectively be back in the scientific middle ages if the enlightenments made possible through recombinant DNA had not occurred.

To my knowledge, moreover, not one case of recombinant DNA-induced illness has since occurred. No person has been so killed, nor has even one case of serious illness been attributed to recombinant DNA, nor do we know of any case where the release into nature of any recombinant DNA-modified organism has led to any known ecological disaster. This is not to say that someday a recombinant DNA-induced disease or ecological upset will not occur. Today, however, there is certainly no logical reason for not exploiting recombinant DNA procedures as fast as possible for human betterment.

**Ideological and Value-based Oppositions to Recombinant DNA Research**

Although there was no evidence of danger from recombinant DNA, there soon arose much visible and sometimes regrettfully effective opposition to recombinant DNA research. Here the distinction should be made between objections from scientists who understand the technical issues involved and opposition from groups of public citizens who, though not understanding the science involved, nonetheless oppose much to all recombinant DNA research. Although some initial opposition arose from scientists whose own DNA research was not going well, virtually all the continuing scientific opponents at their heart had political hang-ups. As leftists, they did not want genes involved in human behavioral differences and feared that the onslaught of scientific advances that would follow from the unleashing of recombinant DNA might eventually allow genes affecting mental performance to be isolated and studied.

As a member of the Harvard Biology Faculty between 1975 and 1977, I watched in despair when "Science for the People" successfully assisted the public members of the Cambridge, Massachusetts, City Council to block recombinant DNA research at our Biological Laboratories. Later, I asked Salvador Luria, who was then at Massachusetts Institute of Technology and who knew that his left-wing friends were putting forth scientifically dishonest statements, why he never publicly criticized them. His reply was that politics was more important than science. This remark has long haunted me, as my own career owes much to the generous way he shared his great scientific talents with me at the beginning of my scientific career. But as a Jew, who had to flee first his native Italy and then France for the eventual safety of the United States, Luria's left-wing political affinities were understandable, and I'm lucky I never had to so choose.

Specific political ideologies, however, are not the cause of the prolonged and sometimes effective opposition to recombinant DNA from parts of the general public, particularly in German-speaking regions. With professional agitators like Jeremy Rifkin playing important roles in heightening these public fears, such leadership would never have been effective if their audiences were at emotional ease with the gene and the geneticists who study it. The concept of genetic determinism is inherently unsettling to the human psyche, which likes to believe that it has some control over its fate. No one feels comfortable with the thought that we, as humans, virtually all contain one to several "bad" genes that are likely to limit our abilities to fully enjoy our lives. Nor do we necessarily take pleasure in the prospect that we will someday have gene therapy procedures that will let scientists enrich the genetic makeups of our descendants. Instead, there has to be genuine concern as to whether our children or their governments decide what genes are good for them.

Genetics as a discipline must thus strive to be the servant of the people, as opposed to these governments, working to mitigate the genetic inequalities arising from the random mutations that generate our genetic diseases. Never again must geneticists be seen as the servants of political
and social masters who need demonstrations of purported genetic inequality to justify their discriminatory social policies. On the whole, I believe that genetics still commands broad respect in the United States and in much of Europe, despite the efforts of the recombinant DNA opponents to portray the genetic manipulations underlying the biotechnology industry as money-driven actions done at the expense of the public’s health and the world’s environment. Unfortunately, genetics and geneticists remain much less highly respected in Germany. There even today the most benign of recombinant DNA experiments remain controversial and subject to needless regulation. Propagation of genetically engineered plants is routinely sabotaged, with the mere practice of human genetics regarded as a criminal act by extremists on the left.

This German dislike for the gene and its human-directed manipulations is easily assignable to their Nazi eugenics past. The vile actions then done in the name of the gene hover as almost permanent nightmares never erasable from their national identity. As human beings, never sure that the world is immune from further such depraved behavior, we should never let this awful past slip from our consciousness. At the same time, the whole civilized world will suffer if today’s German geneticists are unfairly thought to be cut from the same material that clothed those German geneticists, anthropologists, and psychiatrists who not only assisted the Nazi eugenic efforts, but promoted them as scientific-based necessities for German progress.

Part of today’s problem may lie in the postwar fate of Hitler’s biological conspirators. Naively as outsiders we long assumed that they would have all been treated as potential if not real war criminals, with even those of only slight guilt losing all further opportunities for academic existence. But as the German geneticist, Benno Müller-Hill, courageously pointed out in his 1984 book, Todliche Wissenschaft (Murderous Science, Oxford University Press 1988), there was no attempt by the German academic community to find out what truly happened. Instead, it was academically dangerous in Germany to explore the half-truths that allowed many key practitioners of Nazi eugenics to resume important academic posts. A number of professors who early joined the Nazi Party or SS and were directly involved with its genocide programs committed suicide, but there were many Nazi-assisting scientists, successfully claiming that they were only apolitical advisors, who slid quietly back into academic prominence.

The most damning example was that of Professor Otmar von Verschuer, who actively helped the Nazis—first at the Kaiser Wilhelm Institute of Anthropology under Professor Eugen Fischer and later at his own Institute of Human Genetics in Frankfurt. Involved in distinguishing Jews and part-Jews, he later closely collaborated with his former assistant, the now-notorious Joseph Mengele, then doing "scientific" research at Auschwitz. After the war, he nonetheless was appointed to be Professor of Human Genetics at the University of Munster. Equally disturbing was the postwar appointment of Fritz Lenz as head of an institute for the study of human heredity at the University of Gottingen, Germany’s most distinguished university. Although clearly a very competent scientist, he was a major advisor for laws on euthanasia between 1939 and 1941, as well as author of a 1940 memorandum, "Remarks on resettlement from the point of view of guarding the race."

The postwar 1949 exoneration of von Verschuer occurred despite knowledge of the 1946 article in Die New Zeit accusing him of studying eyes and blood samples sent to him from Auschwitz by Joseph Mengele. Yet a committee of professors, including Professor Adolf Butenandt, later the head of the Max Planck Gesellschaft (the postwar name for the Kaiser Wilhelm Gesellschaft), concluded that von Verschuer, who possessed all the qualities appropriate for a scientific researcher and teacher of academic youth, should not be judged on a few isolated events of the past. I find it difficult to believe that the Butenandt committee had gone to the trouble of reading his article published in the Volksicher Beobachter 1-8-42. In it he
wrote, "Never before in the course of history has the political significance of the Jewish question emerged so clearly as it does today. Its definitive solution as a global problem will be determined during the course of this war." Now there may be more reason to remember Professor Butenandt for his part in the von Verschuer whitewash than for his prewar Nobel prize for research on the chemistry of the estrogen sex hormone.

**Genuine Human Genetics Emerges from Recombinant DNA Methodologies**

Long holding back the development of Human Genetics as a major science was the lack of a genetic map allowing human genes to be located along the chromosomes on which they reside. As long as conventional breeding procedures remained the only route to gene mapping, the precise molecular changes underpinning most human genetic diseases seemed foreordained to remain long mysterious. The key breakthrough opening a path around this seemingly insuperable obstacle came in the late 1970s when it was discovered that the exact sequence (order of the genetic letters A, G, T, and C) of a given gene varies from one person to another. Between any two individuals, roughly 1 in 1000 bases are different, with such variations most frequently occurring within the noncoding DNA regions not involved in specifying specific amino acids. Initially most useful were base differences (polymorphisms) which affected DNA cutting by one of the many just discovered "restriction enzymes" that cut DNA molecules within very specific base sequences.

Soon after the existence of DNA polymorphisms became known, proposals were made that they could provide the genetic markers needed to put together human genetic maps. In a 1980 paper, David Botstein, Ron Davis, Mark Scolnick, and Ray White argued that human maps could be obtained through studying the pattern through which polymorphisms were inherited in the members of large multigenerational families. Those polymorphisms that stay together were likely to be located close to each other on a given chromosome. During the next 5 years, two groups, one led by Helen Donis-Keller in Massachusetts, the other led by Ray White in Utah, rose to this challenge, both using DNA from family blood samples stored at CEPH (Centre d'Etude du Polymorphisme Humain), the mapping center established in Paris by Jean Dausset. By 1985, the mutant genes responsible for Huntington's Disease and Cystic Fibrosis (CF) had been located on chromosomes 4 and 7, respectively.

By using a large number of additional polymorphic markers in the original chromosome 7 region implicated in CF, Francis Collins' group in Ann Arbor and L.C. Tsui's group in Toronto located the DNA segment containing the responsible gene. Its DNA sequence revealed that the CF gene coded for a large membrane protein involved in the transport of chloride ions. The first CF mutant they found contained three fewer bases than its normal equivalent and led to a protein product that was nonfunctional because of its lack of a phenylalanine residue.

**The Human Genome Project: Responding to the Need for Efficient Disease Gene Mapping and Isolation**

Although the genes responsible for cystic fibrosis and Huntington's Disease were soon accurately mapped using only a small number of DNA polymorphic markers, the genes behind many other important genetic diseases quickly proved to be much harder to map to a specific chromosome, much less assign to a DNA chromosomal segment short enough to generate hopes for its eventual cloning. All too obviously, the genes behind the large set of still very badly understood diseases like Alzheimer's disease, late-onset diabetes, or breast cancer would be mapped much, much sooner if several thousands more newly mapped DNA polymorphisms
somehow became available. Likewise, the task of locating the chromosomal DNA segment(s) in which the desired disease genes reside would be greatly shortened if all human DNA were publicly available as sets of overlapping cloned DNA segments (contigs). And the scanning of such DNA segments to look for mutationally altered base sequences would go much faster if the complete sequence of all the human DNA were already known. However, to generate these importantly new resources for human genetics, major new sources of money would be needed. So, by early 1986, serious discussions began as to how to start, soon, the complete sequencing of the $3 \times 10^9$ base pairs that collectively make up the human genome (the Human Genome Project or HGP).

Initially, there were more scientific opponents than proponents for what necessarily would be Biology's first megaproject. It would require thousands of scientists and the consumption of some $3$ billion-like sums. Those disliking its prospects feared that, inevitably, it would be run by governmental bureaucrats not up to the job and would employ scientists too dull for assignment to this intellectually challenging research. Out of many protracted meetings held late in 1986 and through 1987, the argument prevailed that the potential rewards for medicine as well as for biological research itself would more than compensate for the monies the Human Genome Project would consume during the 15 years then thought needed to complete it. Moreover, completion of each of the two stages—the collection of many more mapped DNA markers and the subsequent ordering of cloned DNA segments into long overlapping sets (contigs)—would by themselves greatly speed up disease gene isolation.

Always equally important to point out, the 15 years projected to complete the Human Genome Project meant that its annual cost of $200 million at most would represent only 1–2% of the money spent yearly for fundamental biomedical research over the world. There was also the realization that some 100,000 human genes believed sited along their chromosomes would be much easier to find and functionally understand if genome sequences were first established for the much smaller, well-studied model organisms such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster*. Thus, the biologists who worked with these organisms realized that their own research would be speeded up if the Human Genome Project went ahead.

The American public, as represented by their congressional members, proved initially to be much more enthusiastic about the objectives of the Human Genome Project than most supposedly knowledgeable biologists, with their parochial concerns for how federal monies for biology would be divided up. The first congressionally mandated monies for the Human Genome Project became available late in 1987, when many intelligent molecular geneticists still were sitting on the fence as to whether it made sense. In contrast, Congress, being told that big medical advances would virtually automatically flow out of genome knowledge, saw no reason not to move fast. In doing so, they temporarily set aside the question of what human life would be like when the bad genes behind so many of our major diseases were found. Correctly, to my mind, their overwhelming concern was the current horror of diseases like Alzheimer's, not seeing the need then to, perhaps prematurely, worry about the dilemmas arising when individuals are genetically shown at risk for specific diseases years before they show any symptoms.

**Genome Ethics: Programs to Find Ways to Ameliorate Genetic Injustice**

The moment I began in October 1988 my almost 4-year period of helping lead the Human Genome Project, I stated that 3% of the NIH-funded component should support research and discussion on the Ethical, Legal, and Social Implications (ELSI) of the new resulting genetic
knowledge. A lower percentage might be seen as tokenism, while I then could not see wise use of a larger sum. Under my 3% proposal, some $6 million (3% of $200 million) would eventually be so available, a much larger sum than ever before provided by our government for the ethical implications of biological research.

In putting ethics so soon into the Genome agenda, I was responding to my own personal fear that all too soon critics of the Genome Project would point out that I was a representative of the Cold Spring Harbor Laboratory that once housed the controversial Eugenics Record Office. My not forming a genome ethics program quickly might be falsely used as evidence that I was a closet eugenicist, having as my real long-term purpose the unambiguous identification of genes that lead to social and occupational stratification as well as to genes justifying racial discrimination. So I saw the need to be proactive in making ELSI’s major purpose clear from its start—to devise better ways to combat the social injustice that has at its roots bad draws of the genetic dice. Its programs should not be turned into public forums for debating whether genetic inequalities exist. With imperfect gene copying always the evolutionary imperative, there necessarily will always be a constant generation of the new gene disease variants and consequential genetic injustice.

The issues soon considered for ELSI monies were far-ranging. For example, how can we ensure that the results of genetic diagnosis are not misused by prospective employers or insurers? How should we try to see that individuals know what they are committing themselves to when they allow their DNA to be used for genetic analyzing? What concrete steps should be taken to ensure the accuracy of genetic testing? And when a fetus is found to possess genes that will not allow it to develop into a functional human being, who, if anyone, should have the right to terminate the pregnancy?

From their beginnings, our ELSI programs had to reflect primarily the needs of individuals at risk of the oft tragic consequences of genetic disabilities. Only long-term harm would result in the perception of genetics as an honest science if ELSI-type decisions were perceived to be dominated either by the scientists who provided the genetic knowledge or by the government bodies that funded such research. And since women are even in the distant future likely to disproportionately share the burden of caring for the genetically disabled, they should lead the discussions of how more genetic knowledge is to come into our lives.

Human Hesitations in Learning Their Own Genetic Fate

With the initial distribution of American genome monies and the building and equipping the resulting genome centers taking 2 years, the Human Genome Project in its megaphase did not effectively start until the fall of 1990. Decisions to go ahead by funding bodies in the United States helped lead to the subsequent inspired creation of Genethon outside Paris by the French genetic disease charity, Association Française contre les Myopathies (AFM), as well as the building of the now immense Sanger Centre, just south of Cambridge, England, by the British medically oriented charity, the Wellcome Trust. Now effectively 7 years into its projected 15-year life, the Human Genome Project has more than lived up to its role in speeding up genetic disease mapping and subsequent gene cloning. It quickly made successful the search for the gene behind the Fragile X syndrome that leads to severe mental retardation in boys preferentially affected by this sex-linked genetic affliction. The molecular defect found was an expansion of preexisting three-base repetitive sequences that most excitingly increase in length from one generation to the next. The long mysterious phenomenon of anticipation, in which the
severity of a disease grows through subsequent generations, was thus given a molecular explanation. Then at long last, in 1994, the gene for Huntington's Disease was found. Its cause was likewise soon found to be the expansion of a repetitive gene sequence.

While the mapping to a chromosome per se of any disease gene remains an important achievement, the cloning of the disease gene itself is a bigger milestone. Thus, the 1990 finding by Mary Claire King that much hereditary breast cancer is due to a gene on chromosome 17 set off a big gene-cloning race. With that gene in hand, there was a chance that its DNA sequence would reveal the normal function of the protein it codes for. In any case, it gives its possessors the opportunity to examine directly the DNA from individuals known to be at risk for a disease to see whether they had the unwanted gene. Thus, when in 1993 the chromosome 17 breast cancer gene (BRAC1) was isolated by Myriad, the Utah disease gene-finding company, it could inform women so tested for BRAC1 whether or not they had the feared gene.

Initially, concerns were voiced that unbridled commercialization of this capability would all too easily give women knowledge they would not be psychologically prepared to handle. If so, the ethical way to prevent such emotional setbacks might be to regulate both how the tests were given and who should be allowed to be tested. I fear, however, that a major reason behind many such calls for regulation of genetic testing is the hidden agenda of wanting to effectively stop widespread genetic testing by making it so difficult to obtain. Now, however, calls for governmental regulation may fall on increasingly deaf ears. To Myriad's great disappointment, it appears that the great majority of women at 50% risk of being breast cancer gene carriers don't want to be tested. Rather than receive the wrong verdict, they seem to prefer living with uncertainty. Likewise, a very large majority of the individuals at risk for Huntington's Disease are also psychologically predisposed against putting themselves at risk of possibly knowing of their genetic damnation.

Although we are certain to learn in the future of many individuals regretting that they subjected themselves to genetic tests and wishing they had been more forewarned of the potential perils of such knowledge, I do not see how the state can effectively enter into such decisions. Committees of well-intentioned outsiders will never have the intimate knowledge to assess a given individual's psychological need, or not, for a particular piece of scientific or medical knowledge. In the last analysis, we should accept the fact that if scientific knowledge exists, individual persons or families should have the right to decide whether it will lead to their betterment.

Inarguable Existence of Genes Predisposing Humans to Behavioral Disorders

The extraordinarily negative connotations that the term eugenics now conveys is indelibly identified with its past practitioners' unjustified statements that behavioral differences, whether between individuals, families, or the so-called races, largely had their origins in gene differences. Given the primitive power of human genetics, there was no way for such broadly ranging assertions to have been legitimatized by the then methods of science. Even the eugenically minded psychiatrists' claims that defective genes were invariably at the root of their mental patients' symptoms were no more than hunches. Yet, it was by their imputed genetic imperfection that the mentally ill were first sterilized and then, being of no value to the wartime Third Reich, released from their lives by subsequent 'mercy killings.'

But past eugenic horrors in no way justify the "Not in Our Genes" politically correct outlook of many left-wing academics. They still spread the unwarranted message that only our bodies, not
our minds, have genetic origins. Essentially protecting the ideology that all our troubles have
capitalistic exploitative origins, they are particularly uncomfortable with the thought that genes
have any influence on intellectual abilities or that unsocial criminal behavior might owe its origins
to other than class or racially motivated oppression. Whether these scientists on the left actually
believe, say, that the incidence of schizophrenia would seriously lessen if class struggles ended,
however, is not worth finding out.

Instead, we should employ, as fast as we can, the powerful new techniques of human
genetics to find soon the actual schizophrenia predisposing genes. The much higher concor-
dance of schizophrenia in identical versus nonidentical twins unambiguously tells us that they
are there to find. Such twin analysis, however, reveals that genetics cannot be the whole picture.
Since the concordance rates for schizophrenia, as well as for manic-depressive disease, are
more like 60%, not 100%, environmental predisposing factors must exist and, conceivably, viral
infections that affect the brain are sometimes involved.

Unfortunately, still today, the newer statistical tricks for analyzing polymorphic inheritance
patterns have not yet led to the unambiguous mapping of even one major schizophrenic gene to
a defined chromosomal site. The only convincing data involve only the 1% of schizophrenics
whose psychoses seemingly are caused by the small chromosome 22 deletions responsible
also for the so-called St. George facial syndrome. Manic-depressive disease also has been
more than hard to understand genetically. Only last year did solid evidence emerge for a major
predisposing gene on the long arm of chromosome 18. This evidence looks convincing enough
for real hopes that the actual gene involved will be isolated over the next several years.

Given that over half the human genes are thought to be involved in human brain development
and functioning, we must expect that many other behavioral differences between individuals will
also have genetic origins. Recently, there have been claims that both “reckless personalities”
and “unipolar depressions” associate with specific polymorphic forms of genes coding for the
membrane receptors involved in the transmission of signals between nerve cells. Neither claim
now appears to be reproducible, but we should not be surprised to find some subsequent
associations to hold water. Now anathematic to left-wing ideologues is the highly convincing
report of a Dutch family, many of whose male members display particularly violent behavior.
Most excitingly, all of the affected males possess a mutant gene coding for an inactive form of
the enzyme monoamine oxidase. Conceivably having too little of this enzyme, which breaks
down neurotransmitters, leads to the persistence of destructive thoughts and the consequential
aggressive patterns. Subsequent attempts to detect in other violent individuals this same mutant
gene have so far failed. We must expect someday, however, to find that other mutant genes that
lead to altered brain chemistry also lead to asocial activities. Their existence, however, in no way
should be taken to mean that gene variants are the major cause of violence. Nonetheless,
continued denials by the scientific left that genes have no role in how people interact with each
other will inevitably further diminish their already tainted credibility.

Keeping Governments Out of Genetic Decisions

No rational person should have doubts whether genetic knowledge properly used has the
capacity to improve the human condition. Through discovering those genes whose bad variants
make us unhealthy or in some other way unable to function effectively, we can fight back in
several different ways. For example, knowing what is wrong at the molecular level should let us
sometimes develop drugs that will effectively neutralize the harm generated by certain bad
genes. Other genetic disabilities should effectively be neutralized by so-called gene therapy
procedures restoring normal cell functioning by adding good copies of the missing normal genes. Although gene therapy enthusiasts have promised too much for the near future, it is difficult to imagine that they will not with time cure some genetic conditions.

For the time being, however, we should place most of our hopes for genetics on the use of antenatal diagnostic procedures, which increasingly will let us know whether a fetus is carrying a mutant gene that will seriously proscribe its eventual development into a functional human being. By terminating such pregnancies, the threat of horrific disease genes continuing to blight many families' prospects for future success can be erased. But even among individuals who firmly place themselves on the pro-choice side and do not want to limit women's rights for abortion, opinions frequently are voiced that decisions obviously good for individual persons or families may not be appropriate for the societies in which we live. For example, by not wanting to have a physically or mentally handicapped child or one who would have to fight all its life against possible death from cystic fibrosis, are we not reinforcing the second-rate status of such handicapped individuals? And what would be the consequences of isolating genes that give rise to the various forms of dyslexia, opening up the possibility that women will take antenatal tests to see if their prospective child is likely to have a bad reading disorder? Is it not conceivable that such tests would lead to our devoting less resources to the currently reading-handicapped children whom now we accept as an inevitable feature of human life?

That such conundrums may never be truly answerable, however, should not concern us too much. The truly relevant question for most families is whether an obvious good to them will come from having a child with a major handicap. Is it more likely for such children to fall behind in society or will they through such affliction develop the strengths of character and fortitude that lead, like Jeffrey Tate, the noted British conductor, to the head of their packs? Here I'm afraid that the word handicap cannot escape its true definition—being placed at a disadvantage. From this perspective, seeing the bright side of being handicapped is like praising the virtues of extreme poverty. To be sure, there are many individuals who rise out of its inherently degrading states. But we perhaps most realistically should see it as the major origin of asocial behavior that has among its many bad consequences the breeding of criminal violence.

Only harm, thus, I fear will come from any form of society-based restriction on individual genetic decisions. Decisions from committees of well-intentioned individuals will all too often emerge as vehicles for seeming to do good as opposed to doing good. Moreover, we should necessarily worry that once we let governments tell their citizens what they cannot do genetically, we must fear they also have power to tell us what we must do. But for us as individuals to feel comfortable making decisions that affect the genetic makeups of our children, we correspondingly have to become genetically literate. In the future, we must necessarily question any government which does not see this as its responsibility. Will it so not act because it wants to keep such powers for itself?

The Misuse of Genetics by Hitler Should Not Deny Its Use Today

Those of us who venture forth into the public arena to explain what Genetics can or cannot do for society seemingly inevitably come up against individuals who feel that we are somehow the modern equivalents of Hitler. Here we must not fall into the absurd trap of being against everything Hitler was for. It was in no way evil for Hitler to regard mental disease as a scourge on society. Almost everyone then, as still true today, was made uncomfortable by psychotic individuals. It is how Hitler treated German mental patients that still outrages civilized societies.
and lets us call him immoral. Genetics per se can never be evil. It is only when we use or misuse it that morality comes in. That we want to find ways to lessen the impact of mental illness is inherently good. The killing by the Nazis of the German mental patients for reasons of supposed genetic inferiority, however, was barbarianism at its worst.

Because of Hitler's use of the term *Master Race*, we should not feel the need to say that we never want to use genetics to make humans more capable than they are today. The idea that genetics could or should be used to give humans power that they do not now possess, however, strongly upsets many individuals first exposed to the notion. I suspect such fears in some ways are similar to concerns now expressed about the genetically handicapped of today. If more intelligent human beings might someday be created, would we not think less well about ourselves as we exist today? Yet anyone who proclaims that we are now perfect as humans has to be a silly crank. If we could honestly promise young couples that we knew how to give them offspring with superior character, why should we assume they would decline? Those at the top of today's societies might not see the need. But if your life is going nowhere, shouldn't you seize the chance of jump-starting your children's future?

Common sense tells us that if scientists find ways to greatly improve human capabilities, there will be no stopping the public from happily seizing them.

*April 25, 1997*

James D. Watson
DIRECTOR’S REPORT

Throughout many critical periods in the history of the Laboratory, there have been times when a decision to embark on a new research direction has been facilitated by the assembly of a small number individuals who share a common interest. Typically, the new research endeavor has focused around the interests of one or two scientists who provide a complementary approach to a single important problem. Coupled with the recruitment of these young scientists, the Laboratory helps foster interest in the research by establishing courses and meetings so that the new field becomes widely accessible to the broader scientific community. In this way, vigorous new areas of research at Cold Spring Harbor have emerged and more often than not, they have had great impact on biological research in general.

One of the most spectacular examples was the congregation at Cold Spring Harbor in the 1940s of scientists who believed that studying bacteria and the bacterial viruses known as bacteriophages would offer unique insights into the nature of inheritance and of life itself. Made possible by the ravages of war in Europe and the resultant exodus of brilliant young scientists from war-torn countries to the United States, Milislav Demerec (Director 1941-1960) assembled a group of investigators at Cold Spring Harbor who became known as the phage group. Principle among these were Max Delbrück, Salvador Luria, and Alfred Hershey, resident summer scientists at Cold Spring Harbor. Hershey later accepted one of Demerec’s new appointments to the full-time scientific staff. This effective mix of permanent Laboratory faculty and visiting summer scientists ushered in a new era of biological discovery, the precursor to the field now known as molecular biology. Facilitating the expansion of the new research was the establishment of advanced courses at the Laboratory for scientists who wanted to join in on the exciting new opportunities.

A repeat of this phenomena occurred when Jim Watson was appointed Director in 1968 and began to assemble the DNA tumor virus group. The huge expansion of funding for cancer research following the declaration in 1971 of the "War on Cancer" and the exciting opportunities made possible with the ushering in of the recombinant DNA era in 1973 were welcome developments that further boosted research at Cold Spring Harbor. Here again, an interesting mixture of new young staff scientists and visitors to the Laboratory brought new technologies and a research focus that caused a renaissance within the institution. Remarkably, this new research direction was accomplished by the appointment of young scientists who were at the earliest stages of their careers.

Many alumni from that era have gone on to continue their careers throughout the U.S. and numerous other countries as some of the most prominent scientists in the broad field of molecular biology. Most came to the Laboratory immediately after completion of their doctorates and many of these were later promoted to the scientific staff. I was fortunate to have the opportunity to come to Cold Spring Harbor as a Postdoctoral Fellow in 1979 when the tumor virus program was in full swing. Even though some of the earlier scientists had by then moved on to other institutions that wanted tumor virus research of their own, the constant recruitment of young new talent ensured a vigorous research program. The Laboratory was a dynamic and expanding place, with a balance of established, but still young, scientists coupled with newer recruits that hoped to join in on the excitement of the day. I was also happy to be given the opportunity to continue my studies on the replication of adenovirus DNA that were started when I
was a graduate student in Australia, even though no one was at Cold Spring Harbor working on
this topic at the time. The Laboratory was, and remains today an attractive place for Postdoctoral
Fellows to pursue research in an environment that offered fantastic research capabilities and a
large measure of freedom to follow one's interests. Summer visitors in residence also added to
the variety of research life, although with today's dual income families, this aspect of science at
Cold Spring Harbor is becoming all too difficult to continue.

The success of the tumor virus program set the seeds for expansion of the Laboratory into
other research areas that were partially related to the immediate focus of the 1970s. The
philosophy of bringing a number of investigators together to attack a particular biological
problem was repeated many times over. The assemblage of the "yeast group" to study the
control of mating-type and the developmental control of gene expression by DNA transposition
in the late 1970s and the early 1980s resulted in ensuring that the Laboratory was one of the
preeminent institutions in the world that studied yeast genetics. This expansion also helped other
research groups to begin to exploit the power of yeast genetics in their own research programs.
The establishment of the yeast group was preceded by the Laboratory's holding a postgraduate
course on the molecular biology and genetics of yeast that was begun in 1970 by Gerry Fink
and Fred Sherman, a course that is still taught each year. In this case, the course had an impact
on the future science at the Laboratory, rather than the other way around. The completion this
year of the sequence of the entire genome of the yeast Saccharomyces cerevisiae, the first
eukaryotic genome to be sequenced, ensures that this organism will remain an attractive model
for understanding the more complex mammalian cells.

More recently, the outstanding success of the Laboratory's neurobiology research program
that is devoted to understanding cognition derives from decisions in 1990 and 1991 to bring
together young scientists who all have a common interest in learning and memory. Following in
the tradition of the Laboratory, it was also decided to focus on a genetic approach to the
complexity of brain function. There was much concern among our advisors that a genetic
approach to understanding cognition would not work, but our belief in the program and in the
young scientists, coupled with the ability to find the necessary funds to support the
establishment and operating costs over the first critical three or four years, has resulted in a
remarkable program. Just as other successful programs have in the past, we must now ensure
that the learning and memory program can continue to expand and evolve. Part of this can be
achieved by the establishment of new postgraduate courses at the Laboratory to foster new
technologies and expansion of the research to other institutions. This year saw the introduction
of a course on Mouse Behavioral Analysis as an important addition to our rich stable of courses
on neurobiology. This course will couple genetic and behavioral techniques that were foreign to
each other a short while ago.

Our history suggests that the bringing together of a number of talented, young investigators
who share a common interest creates a research environment that transcends the sum of the
individual research programs. It also helps enormously to have scientists who develop their
independent research careers at the Laboratory so that they more easily fit into the Cold Spring
Harbor style of science. It is by no accident that all of our scientific staff were recruited to the
Laboratory at the early stages of their independent research careers. It is also exciting to
witness the development of these individuals and their research programs, some of which will
forever change the Laboratory.

Flexibility to pursue new research areas has been one of the key ingredients to the success
and high productivity of the Laboratory. While we regret that our highly successful scientists get
lured to senior positions in universities, the Laboratory takes the opportunity to appoint bright
young investigators who come here and develop their own programs at the cutting edge of
research. Thus, a healthy mixture of senior faculty positions together with an influx of new blood seems to be essential for our continued success.

In 1986, one aspect of this approach was formalized when the Laboratory began the Cold Spring Harbor Fellow program. Modeled in part after the Junior Fellows program at Harvard, it is intended to offer opportunities for very talented research scientists who have just completed their Ph. D. or M. D. degrees. These unique scientists work at the Laboratory for a period of three years, unencumbered by the normal constraints of formal postdoctoral training in someone else's laboratory. The Laboratory provides salary, technical support, and the necessary funds to support research on a topic of the Fellow's choosing, sometimes in collaboration with existing faculty members, but more often than not, independent of research that involves collaboration. Each of the Cold Spring Harbor Fellows appointed to date has been highly successful. Two former Fellows, Adrian Krainer and Carol Greider, completed highly successful fellowships and were promoted to the scientific staff as independent faculty members. Both are now well respected, senior scientists in their field. Two other former Fellows completed outstanding research at the Laboratory but unfortunately, were then lured to universities; David Barford returned to a faculty position at Oxford University and Eric Richards moved to a faculty position at Washington University in St. Louis. Our initial intention was to have a single Fellow in residence at any one time, but the success of this program suggests that we should expand the number of Fellows in residence. Outstanding students can be nominated for appointment as a Fellow at any time by scientists at universities.

Although tradition has it that recently graduated students will benefit from the ability to perform postdoctoral research in someone else's laboratory, I believe that a few selected students have the ability to continue their graduate studies as independent investigators. The Cold Spring Harbor Fellows program offers this opportunity in a highly supportive research environment. The success of this program is reflected by the fact that this year we appointed two Cold Spring Harbor Fellows, Scott Lowe and Ueli Grossniklaus to the Laboratory faculty, where they will be able to expand their research by recruitment of additional personnel.

This year also saw a reorganization of the structure of the faculty positions at the Laboratory. This was done in part because the new appointments at the Assistant Investigator, Associate Investigator, or Investigator level parallel similar appointments at research universities and the Howard Hughes Medical Institute. Perhaps the most significant change was the appointment of our new faculty to an initial five-year term, rather than the old three-year appointment. Although this provides additional financial burdens on the Laboratory, it also offers these new investigators the time to develop innovative research programs that may take some time to develop. The constant recruitment of new investigators, with their accompanying start-up funds, the need to renovate laboratories and to provide the necessary research equipment, taxes the ability of the Laboratory to support such research, but in the long run, it is these new research areas that often provide the focus for longer term stability.

It is with considerable pride that we reflect on the accomplishments of our alumni who very often made their first important discoveries at the Laboratory. At the same time, the vigor of our current research programs suggest that the Cold Spring Harbor style of doing science continues to be productive.
HIGHLIGHTS OF THE YEAR

Research Highlights

The Tumor Virus Program

Much to my satisfaction, the 1996 review of the Lab's the DNA Tumor Virus Program Project Grant resulted in renewal of this funding for the fifth time. As the term of the grant is five years, we enter our 26th year with this Program Project Grant, good through the year 2001.

This grant was established 25 years ago by Jim Watson and Joe Sambrook to use DNA tumor viruses to study cancer. In 1968, the Lab changed direction toward the study of DNA viruses that infect mammalian cells. Jim recruited Joe Sambrook, among others, to pursue these studies and in 1971 the first application was made for a large ($1 million) program project grant. This program project continues to be a cornerstone of cancer research at the Lab to this day. Over the years this grant has supported the studies of Joe Sambrook, Richard Roberts, Robert Tjian, Michael Botchan, Phillip Sharp, Louise Chow, Earl Ruley, Ed Harlow, Terri Grodzicker, Michael Mathews, Yakov Gluzman, and myself, as well as many others. It was this grant that supported Richard Roberts' studies on split genes that resulted in his winning the Nobel Prize in 1993 with Phillip Sharp.

The Program Project continues to use DNA tumor viruses to probe how normal cells become cancer cells and now includes Winship Herr, Arne Stenlund, Adrian Krainer, Ryuji Kobayashi, and myself. Additional components of cancer research recently added to the grant are studies by Carol Greider into the involvement of telomerase in tumor progression and a multifaceted approach aimed at understanding the mechanisms and regulation of programmed cell death (apoptosis) in cancer cells by Scott Lowe (a former Cold Spring Harbor Fellow), and Yuri Lazebnik. Apoptosis involves a mechanism—built into every cell—that will cause the cell to self-destruct when it is triggered. This process is vital to

![Yuri Lazebnik and Scott Lowe](image-url)
development, ridding organisms of unnecessary cells (i.e., webs from human embryo fingers and toes, and tails from tadpoles as they become frogs) and also by destroying damaged or diseased cells. Most conventional cancer therapies work by triggering apoptosis, and Scott and Yuri each study different aspects of this process.

Scott Lowe reported, early in the year, the discovery of an important characteristic of the p53 gene—a known tumor suppressor. Cells at the center of solid tumors usually die from hypoxia, a lack of oxygen that results from the dense cellular overcrowding caused by the rampant cell division that is characteristic of cancer. Some cells, however, survive in these hypoxic conditions, and Scott, with Tom Graeber and colleagues at Stanford University School of Medicine, has shown that it is the cells with a p53 mutation that survive. Scott had shown previously that cells with a p53 mutation are immune to the effects of conventional cancer therapies. Together these studies provide a link between tumor growth and resistance to cancer therapy; tumor cells which acquire p53 mutations would survive hypoxic conditions and simultaneously become less susceptible to cancer therapy.

Yuri Lazebnik, who studies the mechanisms involved in the execution of programmed cell death, has also focused on drug resistance in cancer cells, and is looking for ways to by-pass this therapeutic obstacle. Because conventional cancer therapy typically triggers apoptosis by damaging the cell to the point that it self-destructs, Yuri is trying to understand why this machinery is not active in some tumor cells despite the presence of the cell death signal. In addition, he is trying to discover how the latent signal in tumor cells can be activated.

Yuri uses an experimental cell-free system that he developed by mixing cytoplasmic extract from a cell with purified nuclei. Howard Fearnhead of Yuri’s lab made an interesting observation in the cell-free system. When he used an extract from normal cells there were no changes to the nuclei, but the addition of an extract from untreated, drug-resistant tumor cells caused apoptotic changes in the nuclei—they began to fragment in the same way as nuclei in dying cells. This indicated that drug-resistant cells have the apoptotic machinery, but they cannot sense the triggering signal that is present. In collaboration with Scott Lowe, Yuri’s lab traced the origin of this signal and found that it is generated by the very same oncogene that transformed the cell to become drug-resistant. Yuri’s lab is now working to further identify the oncogene-generated activity. The hope is that this knowledge could be used to find a way to selectively activate apoptosis in cancer cells.

**Cell Cycle**

Studies of the cell cycle are also an important component of cancer research and this year cell cycle studies in yeast and in mammalian cancer cells complimented one another. Bruce Futcher’s lab has been working to understand how the essential cyclin-dependent protein kinase (CDK) complex (Cdc28-Cln) controls commitment to cell division in yeast, a popular model organism for cancer research. They were particularly interested in how Cdc28-Cln promotes DNA replication. They knew of a protein, Sic1, that inhibits other CDK protein kinases that are necessary for DNA replication. Last year, postdoctoral researcher Brandt Schneider
and graduate student Qing-Hong Yang in the Futcher lab showed that the Cdc28-Cln complex places phosphates on Sic1, following which the phosphorylated Sic1 is attacked by enzymes that digest the protein, thereby allowing DNA replication to begin. In cells that have Sic1, the Cln proteins were essential for replication. A striking observation was that mutant yeast cells lacking Sic1 could commit to DNA replication and cell division even when they lacked the Cln proteins. These results show that the only essential activity of the Cln-like cyclins and the CDK partner CDC28, is to control an inhibitor (sic1) of cyclin CDKs that regulate DNA replication.

These studies complement David Beach’s work on the cell cycle in mammalian cells. Beach’s lab and others have shown that the Retinoblastoma protein (Rb), an important tumor suppressor which inhibits entry into the cell cycle, is itself inhibited by the Cdk4-Cyclin D protein kinase complex—which is similar to the yeast Cdc28-Cln complex. Beach’s lab has found that while Cdk4-Cyclin D is normally essential for DNA replication, it is dispensable in mutants lacking Rb. Furthermore, they have shown that another tumor suppressor protein, p16, regulates Cdk4-cyclin D activity by functioning as a CDK inhibitor.

Many clinical studies have shown that the p16 tumor suppressor is mutated in a wide variety of human cancers. In collaboration with Ron DePinho’s laboratory at the Albert Einstein College of Medicine, the gene that encodes p16 has been mutated in a line of mice so that the mice no longer express the protein. These mice develop tumors of various types at a very high rate, providing proof of the role of p16 as a tumor suppressor. These "knock-out" mice will provide a valuable animal model for the study of human cancer therapy.

**Phosphatases**

In other cell cycle work, David Beach and Konstantin Galaktionov made the important discovery of the regulatory target of the myc onco-gene. That myc is an oncogene has long been known, as has its role in cell cycle: the Myc protein is a driving force in cell division, and in partnership with a protein called Max, can promote either oncogenic transformation (cancer) or apoptosis (programed cell death). Myc is known to be a transcription factor for controlling gene expression, but the critical target genes whose expression it regulates remained obscure. David and Konstantin have found that the cdc25A protein is the target of Myc's function. They were studying Cdc25A because their earlier studies in yeast showed that it was an important CDK regulator, a so-called protein phosphatase. Interestingly, elevated levels of cdc25A have been found in a significant number of breast tumors and other cancers, making these cell cycle components a prime target for anti-cancer drug discovery efforts.

Nicholas Tonks' lab has continued to move forward in their work on the role of protein tyrosine phosphatases (PTPases). These enzymes catalyze dephosphorylation—the removal of phosphate groups from molecules which have been phosphorylated by protein kinases. In 1994, Nick and then-Cold Spring Harbor Fellow and X-ray crystallographer David Barford determined the first three-dimensional structure of a PTPase: the human PTPase 1B (PTP1B). The crystal structure of a
molecule frequently sheds a great deal of light on how the molecule might function—scientists can then see which domains are exposed on the surface, where potential binding sites are located, etc. This information can then be used to develop molecules that effectively block specific actions or interactions. In 1995, Nick and David (by then at Oxford University) built on their success with another "first:" they solved the structure of PTP1B bound to a phosphorylated substrate (the molecule on which an enzyme acts).

In order to accomplish this, Nick and Postdoctoral Fellow Andrew Flint developed a mutant that would bind to its substrate but not dephosphorylate it, and David crystalized and solved the structure of the bound pair. These structures of PTP1B demonstrated that the unique signature motif that is the defining feature of the PTP enzyme family acts as a rigid cradle structure that binds to the phosphate group on a target substrate. The motif lies at the base of a cleft on the surface of the protein and when the substrate binds, the cleft closes, locking the substrate within the enzyme.

These studies are illuminating how a PTPase works; the next question was with what does it work? It has long been thought that there were a limited number of PTPases each of which dephosphorylated a great many substrates. Nick and his colleagues suspected, and have now shown, that PTPases are actually very selective. Together they have developed "substrate-trapping" mutants for a variety of PTPases, like the one used to solve the structure of the PTP1B substrate complex. By expressing substrate-trapping mutants in cells, they can see which substrates the phosphatases recognize. They have made the surprising discovery that many PTPs examined in a physiological context are exquisitely selective, binding consistently to only a small number of substrates. This work has implications for the development of finely targeted therapeutics, as phosphorylation and dephosphorylation are critical to many cellular processes including cell growth, proliferation, and differentiation.

Transcription Control

Another important cellular process is transcription—the formation of a complementary strand of RNA based upon one of the two strands of DNA. But the in vivo study of DNA transcription is difficult because basal transcription factors are vital to cells' survival. Disruption of their function can lead to cell death, making experimentation difficult. Two Cold Spring Harbor scientists, Winship Herr and Nouria Hernandez, study the components of this vital process and their interactions. Some transcription factors, the proteins required to initiate or regulate transcription, recognize the promoter region of the DNA where transcription begins. A key regulatory element in the promoter region of the DNA is known as the TATA box.

Both Winship and Nouria study the TATA box-binding protein (TBP) and its interaction with other transcription factors. Winship's lab developed a new system to study the relationship between TBP and the transcription factor TFIIB in human cells by building on the work of Kevin Struhl of Harvard Medical School. Kevin developed an altered promoter
(TGTA instead of TATA) and an altered TBP binding protein that was engineered to match it. In this system, transcription continued to occur, yet the potentially lethal consequences of disrupting the endogenous transcription machinery were circumvented. Bill Tansey in Winship’s lab took this approach an important step forward: using this altered TGTA box/TBP pair, they developed a new altered pair of basal transcription factors—TBP and TFIIB—that works in concert with the original TGTA box/TBP pair. This "sequential altered specificity" has enabled Winship’s group to study TFIIB in living cells, which was previously not possible in human cells.

Nouria reported interesting results in her work on the interaction between TBP and another group of transcription factors, the small nuclear RNA (snRNA)-activating protein complex (SNAPc), that binds to the U6 RNA polymerase III promoter. TBP consists of a conserved domain long thought to be the only functional region of the protein, while it also contains a non-conserved amino-terminal region long thought to be less, if at all, useful. Nouria has shown that upon truncating the TBP protein to eliminate the amino-terminal domain, the TBP bound much better to the TATA box. In addition, truncation of the amino-terminal domain resulted in strongly diminished binding of SNAPc to its binding site next to the TATA box on the DNA. Her results indicate two previously unknown roles for the non-conserved region of TBP: inhibiting TBP’s binding to the DNA and interacting with another member of the transcription machinery (SNAPc) to promote its binding to the U6 promoter. Hence, the presence of this previously unrecognized component is essential to U6 transcription.

Neurofibromatosis

In cancer studies at Cold Spring Harbor, the ras oncogene, first discovered by Cold Spring Harbor’s Michael Wigler and a group at MIT in 1981, has been the focus of much research. ras is critical to control of growth and development in healthy cells, and when mutated contributes to the formation of tumors. In an exciting interplay between cancer and neuroscience research, a member of our neurobiology team discovered a role for the Ras protein in processes of learning and memory. In related research, the same lab also made an important discovery regarding Neurofibromatosis (NF), a disease that causes learning disabilities in children and tumors in adults.

In 1995 Yi Zhong showed that the ras pathway was a mediating factor in the transmission of certain (G-protein-coupled) neurological impulses. In 1996, Yi’s studies moved toward the investigation of the relationship between ras and the Neurofibromatosis type I (NF1) tumor suppressor gene. NF1 is one of the most commonly inherited neurological disorders in humans. The disease is characterized by tumors (frequently benign), developmental abnormalities, including reduced height, and in many patients (30-45%) specific learning disabilities.

The activity of ras determines the response of cells to a number of signals, including growth factors, that mediate cell proliferation, growth, and differentiation. ras activity can be inhibited by a number of proteins including NF1. Since it is known that elevated ras activity causes cancer
in many human tissues, the diminished inhibition of ras activity in NF patients may well contribute to the development of NF symptoms. However, the story has become even more interesting as Yi Zhong has discovered that the NF1 protein also controls another biochemical pathway in addition to inhibiting the activity of ras. Yi’s lab obtained mutant Drosophila that lacked the NF1 gene from Andre Bernards at the Massachusetts General Hospital in Boston and uncovered a link between NF1 and the cyclic AMP signaling pathway. The latter pathway was previously shown by Tim Tully and Jerry Yin of CSH and others to control learning and memory in Drosophila. Yi found that the NF1 defect could be eliminated by providing small molecules that could activate the cyclic AMP pathway. These results suggest these small molecules as candidate drugs for treatment of NF.

Alcino Silva, who has done important work with mice, has uncovered a mouse model for the study of learning and memory deficits associated with NF. He obtained a line of NF1+/-—mice (one good copy of the gene and one defective) from Tyler Jacks, a collaborator at MIT, and demonstrated learning difficulties similar to those in human NF patients. The development of a mammalian model for the NF disease is facilitating extensive studies of NF that would not be possible in human subjects. These mice will also be valuable in assessing the effects of drugs that affect the cyclic AMP pathway. I am particularly pleased that these exciting results on NF come so soon after the watershed meeting on NF research held at the Banbury Conference Center in late 1995.

In another research project, Alcino continues to study the role of the CREB protein, a target of cyclic AMP, in learning and memory in mice. This year, he had the very exciting success of achieving behavioral rescue in CREB mutant, learning-impaired mice. He demonstrated conclusively that by modifying training regimens for the CREB mutants he was able to overcome profound long-term memory deficits. In these studies Alcino altered the number of training sessions and the length of the rest interval between them. This work follows from the elegant studies on the role of CREB in learning and memory by Tim Tully and Jerry Yin. These examples of successful behavioral rescue through modified training have tremendous implications for the treatment of memory disorders.

Neuronal Learning

In other neurobiology research, Hollis Cline and Roberto Malinow have made great strides in understanding the changes that take place in synapses during development of the neuronal networks and during learning and memory processing. Holly studies the development and stabilization of neurons and synapses while Robert looks at the molecular mechanisms involved in synaptic plasticity—that is, the changes that take place at synapses during learning and the formation of memories.

Holly’s laboratory at CSHL is one of a few laboratories in the world to have succeeded in using confocal microscopy to observe changes in live neurons over the course of several days. She uses high-speed microscopy techniques and Dil, a fluorescent dye, in anesthetized Xenopus frog tadpoles. By watching neurons grow over time, this group has been able to document some remarkable aspects of their development. They saw and photographed developing neurons sending out axon branches
like branches on a tree, but the surprising result of these studies was that most branches extended by the developing neuron were also retracted, in a process of reaching and withdrawing that continued for days, until the cell established a set of extensions with which it was happy. The factors that determine which branches were stabilized in the end remain under investigation.

Once a synapse (a connection between an axon and a receptor cell) is formed, synaptic transmission, the passing of neurological impulses, may begin. Holly and Robert found that newly formed synaptic connections use a type of glutamate receptor called the NMDA receptor. When a synapse matures, it gains a second type of neurotransmitter receptor known as AMPA receptors. They’ve determined that this process is dependent on the entry of calcium into the cell and subsequent activation of the enzyme CaMKII, which in turn stimulates the arrival of AMPA receptors. The exciting result of subsequent experiments showed that when CaMKII is inserted into a developing neuron, the cell then develops characteristics of a mature cell—the branches stabilize and the synapses acquire the second receptor, AMPA.

Robert’s group studies the role of AMPA receptors in a process called LTP, for long-term potentiation. LTP is the strengthening of a neural pathway, a physiological change that takes place with repeated or very strong impulses across a neural pathway. This group studies LTP in the hippocampal region of the rat brain. The hippocampus is a region of the mammalian brain that is implicated in learning and memory. It has been shown that when the hippocampus is damaged, the ability to form long-term memory is impaired; when it is destroyed on both sides of the brain, the ability to form new memories is lost. As LTP is thought to be a critical part of the learning and memory processes, this continued work into the mechanisms of LTP should provide valuable insight into these vital brain functions.

Grigori Enikolopov has expanded on his earlier finding that nitric oxide (NO) acts as a regulator of cell proliferation and differentiation. Initially Grisha and Natalia "Natasha" Peunova showed that NO acts as a molecular switch to tell neuronal precursor cells to stop dividing and to begin developing into, and functioning as, nerve cells. In experiments designed to test the role of NO in other cells in developing Drosophila, Grisha, Natasha, and Boris Kuzin, a visiting scientist from Moscow, have shown that NO also controls organ size during development of an intact organism. Flies in whom the expression of NO was suppressed during larval development displayed substantially larger organs including eyes, wings, genitalia, and legs. The diameter of some leg segments has been 3–4 times that of normal flies, leading to the nickname "Schwartzenegger flies," as they are affectionately known among lab staff.

Tim Tully and Jerry Yin’s quest to isolate new genes involved in the biology of memory was boosted this year by generous support from the Hartford Foundation. The Hartford Foundation is concerned with issues related to aging and is especially enthusiastic about the learning and memory work. The long-term hope of this collaboration is that new genetic information will yield a greater understanding of the causes of aging-related memory dysfunction. As part of this effort, the Hartford Foundation will also support two Banbury meetings—one in 1997 on cognitive functioning and one in 2000 on aging and cognitive dysfunction.
This year, with strong support from Laboratory Board Chairman David Luke III, Cold Spring Harbor scientists Richard McCombie and Rob Martienssen entered into a global collaboration that is determined to sequence the entire genome of a flowering plant, *Arabidopsis thaliana*, by the year 2004. Under a grant of $12.7 million from the National Science Foundation (NSF), the Department of Energy (DOE), and the Department of Agriculture (USDA), three U.S. groups, one European consortium, and one Japanese laboratory have divided up the total genome in order to organize a thorough project with no duplication of effort.

*Arabidopsis* is a popular model organism for plant genetics research. It is a small plant in the mustard family, and has one of the smallest plant genomes and the highest gene density so far identified in a flowering plant.

There are three U.S. groups participating in the project: one is a consortium of sequencing labs headed by Cold Spring Harbor and including groups from Washington University, in St. Louis, Missouri, and Applied Biosystems, Inc., located in Foster City, California. The other two U.S. groups are The Institute for Genomic Research in Rockville, Maryland, and the Consortium of Stanford University, University of Pennsylvania, and University of California at Berkeley. Our overseas collaborators are the European Scientists Sequencing *Arabidopsis* (ESSA), a 17-lab consortium under the direction of Mike Bevan, of the John Innes Center, and the Kazusa DNA Research Institute in Japan, headed by Satoshi Tabata.

The project will result in the first genome sequence of a higher plant and will provide a complete catalog of all the genes involved in the plant life cycle, from seed to flower and fruit. Results garnered by the participants will be made available to the global plant research community almost immediately via the World Wide Web. The resulting knowledge will have agricultural implications for virtually all crops, including wheat and corn, and will be immediately applicable to all economically important plant species.

These numerous and varied good results in our Labs have allowed us to end the year on a very positive tide. As the new year begins, I can already see continued success coming from these research programs.

**Symposium**

The 61st annual Cold Spring Harbor Symposium on Quantitative Biology, held May 29–June 5, marked the sixth time that the Symposium has been dedicated to topics on neurobiology; beginning with The Neuron in 1952, followed by Sensory Receptors in 1965, The Synapse in 1975, Molecular Neurobiology in 1983, and The Brain in 1990, heralding President Bush’s "Decade of the Brain." Each of these meetings has rendered a clearer understanding of the complex nature of the brain and nervous system. This year’s meeting, Function and Dysfunction of the Nervous System, was no exception. Talks focused on integration of neural systems and diseases of the nervous system. Sessions on psychiatric diseases, addiction, neurodegenerative diseases, Alzheimer’s, learning and memory, and neuronal dysfunction indicated strong potential for the application to human disease. This Symposium parallels the Laboratory’s deep commitment to neurobiology.
During the Symposium, the annual Dorcas Cummings Lecture was given by Vilayanur S. Ramachandran, Professor of Neurosciences and Psychology, Director of the Brain and Perception Laboratory, and Co-director of the newly formed Center for Research on Brain and Cognition at University of California, San Diego. His research focuses on an interest in human visual perception and behavioral neurology (the study of patients with focal brain damage). His talk, *Illusions of Body Image in Neurology: What they reveal of human nature*, explored his most recent interest, which examines behavioral correlates of neural plasticity including phenomena such as "phantom limbs," anosognosia or "denial of paralysis," and anorexia nervosa. Most of these syndromes have been known since the turn of the century and treated as enigmatic curiosities on which there has been little research done. Ramachandran has brought them from the clinic to the laboratory and shown that an intensive study of these patients can often provide valuable new insights into the functional organization of the normal human brain. Nearly 300 supporters of the Laboratory, as well as the scientists attending the symposium, attended the lecture.

The Reginald G. Harris Lecture, inaugurated in 1995 and named for the former Laboratory director who initiated the CSH Symposium in 1933, was presented by Richard Axel of the Center for Neurobiology and Behavior at the College of Physicians and Surgeons, Columbia University. His talk entitled *The Molecular Biology of Smell* opened the Sensory Perception session.

On the eighth and final day, Zach W. Hall, Director of the National Institute of Neurological Disorder and Stroke at the NIH, delivered an eloquent and comprehensive summary. He talked about the dual role of Cold Spring Harbor—first, symbolically, as the place where new science begins, and secondly, as a place of unprecedented scientific excitement. His summary was based on two major themes—the link between behavior and biology, and the success of being able to attack brain disease in humans.

**Banbury Conference Center**

*Banbury Center highlights DNA:* The chemistry and biology of DNA are fundamental to so much of our research and to biomedical research throughout the world. It is remarkable that even after so many years of intensive study, we are still discovering novel properties of the molecule and intriguing aspects of its biology. In 1995, Rich Roberts and Xiaodong Cheng, here at the Laboratory, described how an enzyme-DNA methylase that modifies the activities of DNA alters the structure of the double helix in a totally unexpected way. In the same way that an old-fashioned juke box "flipped" a record out of the stack to be played, so the enzyme makes accessible the base it is modifying by "flipping" it out of the helix. The 1996 Banbury meeting DNA Base Flipping: How and Why reviewed new evidence that "base flipping" may not be restricted to DNA methylases but may be a more general mechanism used by other enzymes for interactions with DNA molecules as well.

Banbury Center hosted an historic meeting on the topic of telomerase in 1994. Work in Carol Greider's lab at Cold Spring Harbor and other laboratories has shown that telomere replication may play important roles
in both cancer and aging. Telomerase is the enzyme that is used by the cell to sustain telomeres (the ends of chromosomes) and this field has become a very "hot" area of research. The follow-up meeting in 1996, Telomeres and Telomerase, drew many of the participants of the 1994 meeting together with researchers new to the topic. The 1996 meeting, like the earlier meeting, provided an opportunity for the presentation and discussion of new data, unpublished and controversial.

One of the most remarkable meetings of the year dealt with one of the oldest problems in molecular biology: how RNA molecules are made from the appropriate genes and at the appropriate times. The field has reached a stage of development where critical review and synthesis are needed, and the Banbury meeting Mechanisms of Transcriptional Initiation succeeded in providing at least the critical review. The meeting was notable for its organization, the schedule being developed as the meeting progressed, with speakers being limited to just a few minutes and just a few slides.

**The Executives' Conference**

This series of meetings continues to be a pleasant surprise. Each year sets such high standards for the following year, that it is not clear how we will be able to find new topics of sufficient interest. It is a measure of the vitality and vigor of biomedical research that new subjects are continually appearing. Human Development—a topic much in the news now with the cloning of a sheep—was the topic for 1996, and covered human embryonic development, the development of gender differences, and the fertilization and manipulation of human embryos in vitro.

**Education for Nonscientists at Banbury Center**

Among the most influential meetings held at Banbury Center were those funded by the Alfred P. Sloan Foundation for workshops on biomedical research for Congressional staff and science journalists. In 1996, the Federal Judicial Center in Washington, D.C. brought federal and state judges to Banbury to learn about many aspects of science in the courtroom. Topics ranged from the history and philosophy of scientific practice to issues of direct application, such as the use of statistical evidence and how courts understand risk assessment. Genetics was not neglected, with talks on eugenics, genetics, and social implications of the Human Genome Project. One issue that was raised repeatedly concerned expert witnesses and how to establish their credibility in litigation where fees for testimony speak very loudly.

In November, the Banbury Center hosted a workshop for science journalists similar in format to previous meetings funded by the Sloan Foundation. This time supported by funds from the Lab’s Department of Public Affairs, the title of the meeting was Genetics of Human Behavior. Talks addressed genetic factors involved in such "behavioral" issues as alcoholism, homosexuality, aggressive behavior, and learning and memory. The meeting was a stimulating and educational experience for more than 20 science writers.
Board of Trustees

Individual trustees Charles Dolan, Owen Smith, and Henry Wendt as well as scientific trustee Shirley Tilghman each concluded a full term on the Board in 1996. I look forward to continued advice and interaction with these friends and colleagues.

Mary D. Lindsay, a friend and advocate for more than 40 years and a board member as often as the bylaws allow since 1971, has concluded her most recent term. She had been a member of the Executive Committee since the early 1980s and since 1992 was vice chairman of the Board. Over the years, Mary has served on most board committees. Among her longest and most valued tenure was her active role on the Building Committee. In November, the Board elected Mary Lindsay an honorary trustee for life.

Most recently Mary's long-held commitment to the young scientists and their families came to fruition in the establishment of child care here. Ground breaking, demolition and renovation of the De Forest Stables has begun to make way for what will become a fine facility to serve the youngsters of our staff.

The election of five new individual trustees and one scientific trustee in 1996 continues the strong board participation that the Laboratory has grown to depend on. C. Thomas Caskey, M.D., senior vice president of research for Merck Research Laboratories in West Point, Pennsylvania, not only brings his extensive research experience in mammalian genetics and inherited diseases but his astute business acumen in the area of biotechnology. Helen Dolan, longtime resident of nearby Oyster Bay, continues to represent the ever charitable Dolan family, who continue to be close friends of the Laboratory. Lola Grace is a financial consultant specializing in financial institutions and has already made a tremendous contribution with her outstanding leadership of the Emanuel Ax Gala in October and her avid encouragement of Lab partnerships with local private and public schools. Lola continues the strong association between the Grace family and our Board. Leon Polsky, a lawyer residing in New York City, has extensive legal and judicial experience to bring to the Laboratory. Mr. Polsky is the son-in-law of the late, revered trustee Lita Annenberg Hazen. William Matheson, retired since 1992 after practicing law in New York City for 42 years, has established with Mrs. Matheson the Matheson Endowment for Neuroscience. The Mathesons make their home in Florida.

I am happy to welcome once again Dr. Arnold Levine, who served as a trustee from 1976–1980. Codiscoverer of the important p53 tumor suppressor protein, he studies virus-induced oncogenesis and cancer biology at Princeton University in the Department of Molecular Biology.

To trustee and dear friend Wendy Russell, we all extend our heartfelt sympathy at the loss of her beloved husband Bill Russell. In the short time that we knew Bill, it was clear that he had fast become the same avid proponent of the Laboratory as is his enthusiastic wife.

Robertson Research Fund

The Robertson Research Fund was established by the Robertson family of Lloyd Harbor in 1973. The initial gift from Charles Robertson was nearly $8 million and coincided with the transfer of the Robertson property on
Banbury Lane to the Laboratory. The site of our Banbury Conference Center, this property is further supported by a maintenance fund, also created by the Robertson family.

Two years later in 1975, the Banbury Fund—the Robertson family’s private foundation—established the Marie H. Robertson Memorial Fund to support neuroscience research at Cold Spring Harbor. This year, the Marie Robertson fund distributed $125,000 to support Tim Tully and his work on learning and memory in Drosophila; a new postdoctoral researcher for Alcino Silva, who studies learning and memory in mice; two neurobiology seminars (given by Josh Gordon of University of California, San Francisco and Menahem Segal of the Fogarty International Center in Bethesda, Maryland); and a Banbury Meeting on Genetic Approaches to Learning and Memory.

In 1996 the Robertson Research Fund—its value now up to $59 million—distributed $1.8 million to basic research at Cold Spring Harbor. These funds provided research program support directly to 20 scientists; very important supplemental support for the Lab’s postdoctoral fellows; and support for graduate students and our seminar program. These most generous gifts continue to have a monumental impact on science at the Laboratory.

Major Gifts

The Laboratory’s research programs received strong support in 1996. Plant research received a second substantial gift from Laboratory Board Chairman David L. Luke III and his wife Fanny, who gave $362,250 to Arabidopsis research. In 1995, Mr. Luke and Westvaco Corporation provided seed money of $290,000 to establish a plant sequencing project at Cold Spring Harbor. This gift allowed the Lab to become a major player in an important international collaboration to sequence the genome of the plant Arabidopsis thaliana. In additional plant research support, Pioneer Hi-Bred International, Inc. gave $80,000 to support Research Investigator Joseph Colosanti.

The Arnold and Mabel Beckman Foundation supported structural biologist Leemor Joshua-Tor with a $200,000 new investigator grant; the Mellam Family Foundation provided $200,000 to Nick Tonks for his work with PTPases; the Pew Scholars Program in the biomedical sciences gave $200,000 to Tatsuya Hirano in a four-year scholarship for research on the dynamics of chromosome structure; the Oliver S. & Jenny R. Donaldson Charitable Trust gave $125,000 to Michael Hengartner for his work on programmed cell death; the Swartz Foundation gave $77,274 to help establish a computational neurobiology research program at the Laboratory; the Charles A. Dana Foundation supported Tom Marr in his research into the genetic basis of manic-depressive illness with $194,000; while Glaxo-Wellcome, Inc. gave $100,000 for postdoctoral fellows working on cell cycle and apoptosis in laboratories headed by Yuri Lazebnik, Michael Hengartner, and David Beach. 1 in 9: The Long Island Breast Cancer Action Coalition gave $50,000 to Michael Wigler’s lab for breast cancer research; Mr. and Mrs. Alan Seligson gave $35,000 to the ongoing Andrew Seligson Memorial Fellowship; the Goldring Family Foundation gave $30,000 for a Postdoctoral Fellow in my laboratory; Oxnard Foundation donated $20,000 to muscular dystrophy research;
and the Lauri Strauss Leukemia Foundation granted $15,000 to Nick Tonks through their Felix Schnyder Memorial Fund.

Gifts to neuroscience research were numerous. We are particularly grateful to Marjorie A. and William L. Matheson for establishing The Matheson Endowment Fund for Neuroscience with gifts totaling $2,869,227. The John A. Hartford Foundation gave $320,082 to Tim Tully; Lita Annenberg Hazen Foundation provided $200,000 to Neurobiology research; the McKnight Endowment Fund for Neuroscience gave $150,000 to Jerry Yin, and the G. Harold and Leila Y. Mathers Charitable Foundation donated $126,750 to Robert Malinow. New York Community Trust gifted $60,000 to Alcino Silva, and the National Neurofibromatosis Foundation granted Alcino $50,000, while the Volkswagen Foundation gave him $32,808. Both the Helen Hoyt Foundation gift of $20,000 and the Eppler Foundation for Research gift of $15,000 went to support Holly Cline's research into neuronal growth.

Badly needed equipment money was received from The Oliver S. & Jenny R. Donaldson Charitable Trust, who gave $100,000 to Dick McCombie for an automated DNA Sequencer (used in both cancer and plant research), William and Maude Pritchard Charitable Trust who gave $75,000 for neurobiology equipment, and the Slocum Estate gave $8,000 for other essential equipment.

In the earliest stages of fund-raising for the Cold Spring Harbor Laboratory Advanced Imaging Facility and associated start-up costs, we received in 1996 generous commitments of $300,000 from the William Stamps Farish Fund and $250,000 from the Gladys and Roland Harriman Foundation. The Lucille P. Markey Charitable Trust gave $500,000 to Karel Svoboda, a young neurobiologist who will join us in 1997 as we begin our new imaging effort.

The Emanuel Ax Gala was the most successful fund-raiser in Lab history. Major gifts to this event include the Anderson Group, $10,000; Mr. and Mrs. Charles Dolan, $10,000; Mrs. Oliver R. Grace, $10,000; Mr. and Mrs. John S. Grace, $5,000; J.P. Morgan and Co., Inc., $5,000; Mr. and Mrs. Edwin S. Marks, $5,000, Mr. and Mrs. William R. Miller, $5,000, Mr. and Mrs. Thomas A. Saunders III, $5,000, Mr. and Mrs. Douglas A. Soref, $5,000. A complete list of contributions to the gala may be found in the financial section at back.

Donations to the Second Century Endowment Fund included $393,000 from the estate of Eric Ridder, $50,000 from the Banbury Fund, $49,960 from Henry Wilmerding, and $43,871 from Robert L. Garland by way of a contribution to the pooled income fund.

Carol Large, chairman of the capital campaign for the Mary D. Lindsay Child Care Center held a marvelous kick-off luncheon on September 18 for a very enthusiastic committee. By the end of 1996, more than $620,000 had been raised. Board members, CSHL Association directors, and members of the committee were responsible for contributing nearly half toward the $1-million goal. Individuals and foundations have shown their support for Lab families with generous donations: Edwin Marks, $185,553; The Weezie Foundation, $75,000; Mr. and Mrs. Robert G. Merrill, $54,380; Anonymous, $50,000; Mr. and Mrs. David L. Luke III, $50,000; Mrs. John H. Livingston, $26,468; David H. Koch Charitable Trust, $25,000; Mr. and Mrs. James M. Large, $20,000; Dr. and Mrs. James D. Watson, $20,000; Schiff Foundation, $15,000; Dickey Family,
$12,000; Mr. and Mrs. Peter O. A. Solbert, $10,171; G. Morgan Browne, $10,000; Hyde and Watson Foundation, $10,000; Mr. and Mrs. Robert V. Lindsay, $10,000; Mrs. Donald A. Straus, $10,000; Mr. and Mrs. Howard Phipps, Jr., $10,000; Mr. Arthur C. Merrill, $7,500; Mr. and Mrs. Henry Wendt, $7,500; Mr. and Mrs. John P. Cleary, $5,000; Mr. and Mrs. Edward H. Gerry, $5,000; and Mr. and Mrs. Robert D. Lindsay, $5,000. A complete list of donors may be found in the financial section of this report.

DNA Learning Center
The DNALC received its 100,000th visitor in 1996. While this would be a yearly or even quarterly statistic for a large science center, the numbers alone do not tell the whole story. Half of all visitors, overwhelmingly precollege students from the New York metropolitan area, participated in a two-to-three hour laboratory experience. Visitation has tripled since opening in 1988—to 22,720 students, teachers, and families in 1996. Also over this period, the lab clientele has shifted from almost entirely high school students to more than half middle school students. Lab field trips were increased by 30% in 1996 by adding more afternoon sessions for middle school students. However, with double or triple booking virtually every school day between October 1 and June 15, the Bio2000 Laboratory has reached saturation. The DNALC’s educational reach was further expanded with the opening of its own World Wide Web site (http://darwin.cshl.org). By year’s end the site was receiving more than 10,000 "virtual" visitors per month, including representatives from more than 50 countries.

In September, the DNALC celebrated the opening of the McClintock Exhibit, a tribute to the late Barbara McClintock and the Nobel Prize-winning work that she did during five decades at Cold Spring Harbor. The display consists of a recreation of Barbara’s laboratory bench and includes original equipment, books and personal effects that had been archived since Barbara’s death in 1992. It also includes a biography, her awards, and a video tape of Barbara’s acceptance speech at the Nobel Ceremony in Stockholm in 1983.

The enthusiasm for DNALC programs was matched by the exceptionally strong support of Long Island businesses represented on the Corporate Advisory Board. This body, chaired by John Leahy of Chase Manhattan Bank, raised $147,000 in support of the DNALC’s programs for local students. This contribution equaled 17% of 1996 operating costs. Looking toward the DNALC’s 10th anniversary in 1997, we will have to consider ways to insure that it maintains its position as the world’s leader in gene education. This will include enlarging the DNALC facility, expanding genetic information available at its WWW site, and convincing the state government to accept some responsibility in providing annual support for this unique New York resource.

Scientific Honors
Carol Greider and her work on telomerase have received considerable recognition in recent months. In addition to a good deal of attention from the popular press, Carol has been lauded by several prestigious organi-
Winship Herr (right) accepts Laboratory's award from Herbert Zaretsky, American Cancer Society

Carol was awarded the American Society for Cell Biology's (ASCB) first Glenn Foundation Award. The American Society for Biochemistry and Molecular Biology (ASBMB) honored Carol with the Schering Plough Award, an award reserved for researchers who are within ten years of receiving their Ph.D. Perhaps most exciting, and indeed challenging, was her appointment by President Clinton to the newly established National Bioethics Advisory Commission (NBAC). This appointment came just in time to allow Carol to be a guiding force in deciding how the Clinton administration will deal with ethical issues related to the cloning of mammals.

David Beach, highly respected for his work in understanding control of the cell division cycle and its implications for cancer, was elected this year a Fellow of the Royal Society—the United Kingdom's prestigious national academy of science. Founded in 1660, the Society promotes improvements in natural knowledge. David attended the admission day ceremony in London on June 6 and received the certificate of fellowship.

Michael Hengartner, a young apoptosis researcher, was awarded the Pharmacia Biotech Prize for Young Scientists. Sponsored by the American Association for the Advancement of Science (AAAS), this honor recognizes outstanding molecular biologists at the earliest stage of their careers.

Structural biologist Leemor Joshua-Tor, who arrived at Cold Spring Harbor in late 1994, received the Beckman Young Investigator Award. Leemor uses X-ray crystallography and biochemistry to study the structure and function of cellular proteins and enzymes.

And finally, the Laboratory was honored when the Long Island division of the American Cancer Society bestowed its special Excellence in Research Award on the Laboratory for its "leading edge research program in cancer biology."

Cold Spring Harbor Laboratory Press

Eight books and two videos were published this year. Notable new titles included the Symposium volume on Protein Kinesis and two new volumes in the CSHL Monograph series. DNA Replication in Eukaryotic Cells, edited by Melvin DePamphlis, is composed of three sections (concepts, proteins, and systems) so it serves as an introduction to this important topic for scientists-in-training and as a core reference work for experienced investigators. Epigenetic Mechanisms of Gene Regulation, edited by Vincenzo Russo, Robert Martienssen, and Arthur Riggs, discusses our current understanding of the observation first made by Barbara McClintock in maize, that heritable changes in gene function cannot all be explained by changes in DNA sequence. The mechanisms underlying these phenomena are reviewed in the many species in which they are now known to occur. Also notable was the reissue of an expanded edition of Horace Freeland Judson's absorbing history of the development of molecular biology, The Eighth Day of Creation. The additional material in this edition includes challenging essays on the lives and scientific contributions of Rosalind Franklin and Erwin Chargaff.

In December, Nancy Ford, the book program's managing editor, announced her decision to retire after 24 years' of extraordinary service to the Laboratory. She played a leading role in the creation of the book.
publishing program at Cold Spring Harbor and is remembered with affection and admiration by many of the authors and editors with whom she produced so many outstanding contributions to the scientific literature.

The journal program continued to strengthen. All three journals had increased circulation and the impact factor of *Genes & Development* rose for the eighth consecutive year, placing it among the six most influential science journals in the world.

**Cold Spring Harbor Laboratory Association**

The Cold Spring Harbor Laboratory Association, under the skillful leadership of President John Cleary, again enriched the Laboratory with its many activities. Three dynamic and enthusiastic directors were elected to the CSHL Association in 1996: Trudy Calabrese, Tony Kemper, and Cathy Soref. Tony and Cathy have contributed greatly to the growth and continued success of the Next Generation Initiative (NGI) while Trudy put her efforts toward raising funds for the capital campaign for our child care center and developing new contacts for our annual membership drive.

The NGI program has generated new and renewed interest in the Lab. The focus of this program is to encourage people in their thirties and forties to become interested in science at the Lab and to encourage new membership in the CSHL Association. At an NGI lecture on January 19, 1997, I presented an overall view of our research program and history and Tim Tully described the newest findings in learning and memory in *Drosophila*. Following the program, five private dinner parties were given, echoing the very successful dinner parties held annually since 1954 following the Dorcas Cummings lecture.

The traditional and highly regarded Symposium Dinner Parties were a notable success under the 1996 leadership of Vernon Merrill, a director of the Association. Twenty-two hosts and hostesses opened their homes to personal guests, visiting and Lab scientists for an evening of exceptional food and lively intellectual exchange.

The Association’s annual membership total of well over $600,000 was gratifying in light of the fact that a major capital campaign for the child care center and the Emanuel Ax benefit were under way concurrent with the fall membership drive.
Allen Oliff, M.D., executive director of Cancer Research at Merck Research Laboratory, addressed the Association members at their Annual Meeting in February. His lecture, *Anti-cancer Agents Directed Against Oncogene Targets*, provided an enlightening discussion of the long and complicated process involved in turning basic scientific knowledge into therapeutic clinical treatments.

The Association orchestrated a variety of other special events during the course of the year. On May 5, a concert by pianist and writer, Carol Montparker, was complimented by an exhibition of the works of Stan Brodsky, noted artist and teacher. This provided a double treat for the Association members and friends who attended.

On October 13, our good friend Dr. Cynthia J. MacKay and Dr. Peter Gouras, her collaborator from Columbia-Presbyterian Hospital, presented a much-needed discussion of macular degeneration, the number one cause of legal blindness in people over 65 in the United States. The lecture drew an inquisitive audience to hear about retinal cell transplantation, a new treatment for this debilitating affliction. A lively question and answer session followed; this lecture made a valuable contribution to our friends and neighbors.

On November 17, 1996, Dr. David Grimaldi, Curator and Chairman of the Department of Entomology at the American Museum of Natural History, presented an informative lecture on *Amber: A Scientific Renaissance*, to a packed house of adults and children. His focus was on both the history and biology of this ancient resin. Afterward, Dr. Grimaldi remained in the lobby to sign copies of his book, *Amber: Window to the Past*, and to continue answering individual questions from the guests about their own pieces of amber.

Also in November, this year’s Major Donor Cocktail Party was graciously hosted by former Laboratory trustee Owen Smith and his wife Bernadette in their beautiful home, *Whispering Laurels*, for major donors and Laboratory scientists.

**President’s Council**

The President’s Council was formed three years ago in an effort to bring together a small group of individuals interested in science and the work
at Cold Spring Harbor Laboratory. Members of the President's Council provide support for the Cold Spring Harbor Fellows program through an annual commitment of $25,000. This funding is critical to the Lab's continued initiative to attract top young scientists fresh from their Ph.D. studies. These fellowships allow new researchers to pursue their own research, rather than assisting in the laboratory of an established scientist.

A major feature of the Council is an annual meeting that brings together this select group of leaders from business, finance, and science to discuss the latest developments in genetic research and biotechnology. The Council's 1996 meeting, held May 17-18, commenced with lunch on Friday at Ballybung, the home of Jim and Liz Watson, and was followed by thought-provoking lectures on learning and memory by Drs. Tom Marr and Alcino Silva of Cold Spring Harbor Laboratory. The keynote speaker, Dr. Barry Gordon, Associate Professor of Neurology and Cognitive Science at John Hopkins University Medical School, opened the evening session with his talk on Remembering and Forgetting. Saturday's highlights included lectures by Dr. Eric Kandel, Howard Hughes Medical Institute and Columbia University; Dr. Tim Tully, Cold Spring Harbor Laboratory, Dr. Paula Tallal, Center for Molecular and Behavioral Neuroscience, Rutgers University; and Dr. Earl Hunt, University of Washington. The mix of minds—leaders in the business world and in the scientific community—evoked interesting insights as well as provocative discussions. The meeting ended on Saturday with the guests gathering once again at Ballybung for a parting luncheon.

Members of the President's Council include Christopher Alafi, Alafi Capital; Abraham Appel, Appel Consultants; Peter L. Bloom, General Atlantic Partners; James T. Conneen, A.T. Hudson & Co.; Michel David-Weill, Lazard Freres & Co.; Frederick Frank, Lehman Brothers, Inc.; Leo A. Guthart, ADEMCO; Charles E. Harris, Harris & Harris Group, Inc.; Walter B. Kissinger, Long Island Research Institute; Donald A. Pels, Pelsco, Inc.; George B. Rathmann, ICOS Corporation; Hubert J. P. Schoemaker, Centocor, Inc.; James H. Simons, Renaissance Technologies Corp.; and Sigi Ziering, Diagnostic Products Corporation.

A Very Special Benefit

On October 13, 1996 renowned concert pianist Emanuel Ax provided a most memorable evening. In addition to surpassing all previous events in its success as a fund-raiser, the evening was a spectacular musical, culinary, and visual experience. The outstanding performance of Mr. Ax and a formal dinner for the 350 attendees were enhanced by the dramatic decorations brought in for the event. Shelley Galehouse King Broven's faux art added the finishing touches. Gala Chairman Lola Grace, with co-chairmen Hope Lapsley, Carol Large, and Cathy Soref and a committee of 75 pulled off a stellar event, which raised over $134,000, with great style.

Concerts

Grace Auditorium was the setting for many other musical performances during our meeting season. On April 27, at the meeting on zebrafish,
pianist Max Levinson performed a moving and enjoyable piano recital. This concert was sponsored by a gift from Dr. Mark Ptashne.

Young Artists concerts included violinist Joseph Lin on August 17, accompanied by Benjamin Loeb on piano and held during the Cancer Genetics meeting. On August 24, Todd Palmer, clarinetist, and Margaret Kampmeier, pianist, performed during Bacteria and Phage, and Mikhail Yanovitsky gave a piano concert on September 7 during Translational Control. In addition, Andrew Russo, an invited guest of Liz Watson, performed a piano recital in the intimate Davenport Music Room on September 22.

Child Care Realized

A long-held desire to provide child care to our staff is finally a reality. Several hurdles were cleared in 1996—the Village Board approved our plan for the renovation of the DeForest Stables at the north end of Bungtown Road. A ground breaking on June 23, complete with golden shovel and balloons, was attended by Village and Laboratory Trustees as well as our Laurel Hollow neighbors and members of our extended Lab family. Among the speakers were Sheri Lucero from Centerbrook Architects and Mimi Leshin, recently retired director of the Long Island/National Employee Assistance Providers Inc., and an expert on child care in the workplace.

A Laboratory committee, chaired by Cheryl Sinclair, director of human resources, comprised of scientists, trustees, staff, and outside experts, was charged with recommending appropriate child care. After exhaustive research which included impromptu site visits, interviews and comparative proposals, the committee recommended the selection of Rainbow Chimes, Inc. This child care provider was established in 1980 under the leadership of Kathleen Roche, R.N., founder and executive director and Laura Ludlum, associate director since 1985. Rainbow Chimes provided site consultation, family orientation, and workshops for youngsters both here at the Laboratory and at their location in Huntington. Kathleen Roche and her team bring the right kind of flexibility and quality needed by our Lab families. The Mary D. Lindsay Child Care Center will be open
to our infants, toddlers and pre-schoolers in summer 1997. The dedication is planned for June 21, 1997.

**Gavin Borden Visiting Fellow**

The Gavin Borden Visiting Fellow pays tribute to the late Gavin Borden, founder of Garland Publishing. Included in Garland's list are textbooks for graduate students, the most successful of which is the popular *Molecular Biology of the Cell* by Alberts et al. The Borden Fellow is chosen each year by our graduate students, and speaks to and visits with the students and staff. This year's Visiting Fellow was Martin Raff, an author of *MBC* and professor of biology at the University College of London. He opened his talk with warm reflections on time spent with Gavin Borden, who died most prematurely at only 52 years of age, then went on to offer an eloquent discussion of the control of cell numbers during development. Using the central nervous system as an example, glial cells specifically, Raff explored the many components that must be in place for proper control of cell division and differentiation. The evening lecture was followed by a dinner in the Clarkson dining room. The following day, Dr. Raff met in seminar with the graduate students.

**Undergraduate Research Program**

The Laboratory's summer Undergraduate Research Program (URP), initiated in 1959, has matriculated 462 students, affectionately known as URPs. Many have gone on to productive careers in the biological sciences. The program exposes students to hands-on experimental approaches to science and to a greater degree of understanding of the issues involved in biochemistry, genetics, and in molecular and cellular biology.

In 1996, administration of this program passed from Assistant Director Winship Herr, who ran the program for the last 10 years, to scientist Michael Hengartner.

Twenty-two students were chosen from more than 280 applicants for this year's Undergraduate Research Program. On March 7, 1996 the Lab received a $1 million gift to the URP endowment from Burroughs Wellcome. In addition to the Wellcome Fund, the 1996 program was supported by Jephson Educational Trust, Cornelius N. Bliss Memorial Fund, Share-It-Now-Foundation, Nathan Springer, Donald J. Sutherland, Dr. Ira Herskowitz, Angus P. McIntyre, David B. Kaback, and the Qosina Corporation. A list of the students, their schools, mentors and research projects may be found in the Undergraduate Research Program section of this annual report.

**Partners for the Future (PFF)**

Through this program, designed to introduce high school students to the world of basic research, the Lab welcomed five teenagers again this year. The students work October through March with scientist mentors from the Lab, on original research projects. I am pleased to welcome Cablevision as the first corporate partner for the PFF program, and am
grateful for their forethought in establishing this partnership. This year their corporate sponsorship funded Sophia Virani of Herricks High School, who worked with Hong Ma on plant genetics. The other participants, their high schools, and Lab mentors are: Jana Steiger, Oyster Bay High School (with Peter Nestler); David Nussbaum, Half Hollow Hills High School West (Michael Regulski); Robert Wlodarczyk, Hauppauge High School (John Connolly); and Maddalena Pizzirusso, Sewanhaka High School (Erich Grotewold).

Project WISE
For the second consecutive year, Cold Spring Harbor Laboratory took part in Project WISE—Women in Science and Engineering. Orchestrated by SUNY Stony Brook and funded by a grant from the National Science Foundation, the project involves several Long Island institutions—SUNY, CSHL, Brookhaven National Laboratory, and the American Association of University Women—each helping to expose bright young women to the world of science. The program for female high school students requires the student’s participation during each of her four high school years—9th through 12th grade. The Lab instructs the 10th graders in molecular biology and genetics, offering a foundation upon which they can build a future in technology and the sciences.

Mary Horton of our Grants Department ably managed our involvement in this ambitious program, which includes two trips to the DNA Learning Center, dinners with Lab scientists, and visits to CSHL labs. Mentors in 1996 were staff investigators Holly Cline and Michael Hengartner; post-doctoral fellows Karen Buchkovich, Alyson Kass-Eisler, Michele Cleary, and John Horton; and research technician Stephanie Smith.

Educational Outreach
The Laboratory’s responsibility to local private and public schools continues to expand through Lab tours, nature walks, chats on the internet with and lectures by Lab scientists.

Eastwoods School pre-K children visited Xiaodong Cheng’s X-ray crystallography lab, the third grade learned about Tim Tully’s flies, and their eighth graders worked with combinatorial chemist Peter Nestler and postdoctoral researcher John Horton. In addition, each class took at least one historical tour and an annual hunt along our beaches. The Inter-school Exchange, a consortium of local private schools made up of Eastwoods, Portledge, Friends Academy, Holy Child, Greenvale and Buckley Country Day School, is a group who shares community resources. CSHL Association director Cathy Soref has been coordinating the Lab’s contribution to this group as a scientific resource. As well, Friends Academy shared a special evening program with Lab staff Alyson Cass Eisler, Brandt Schnieder and Mona Spector which included e-mail follow up with those scientists.

For West Side School, within the Cold Spring Harbor School District, we conducted three Science Nights. At each event a CSH scientist addressed a group of elementary school children and their parents and teachers. On April 1, visiting scientist Sandy Williams presented Heart Attack? Genes to the Rescue. On May 20, Alcino Silva talked to a group
about learning and memory in mice, and Carol Greider ended the series on June 17, discussing You and Your Chromosomes.

For the second year, we cooperated with Cold Spring Harbor High School as they hosted a group of Japanese high school students and their teachers. The group spoke little English, but worked with a Japanese English teacher as translator. When the vocabulary for a scientific tour of Cold Spring Harbor Lab was beyond the translator’s vocabulary, we were fortunate to have the help of research investigators Akila Mayeda and Shou Waga, and postdoctoral researcher Kyoko Hidaka, who discussed our history, science, and educational programs with the group.

Other tours included Westbury Friends School (Westbury), Sacred Heart Academy (Hempstead), Greenvale School (Glen Head), and assorted other schools and universities. For each of these tours we ask two or more scientists to take a break from their research and show the students and teachers around their laboratory. Our scientists are gracious hosts to these groups and their participation enriches the experience for our visitors.

Winterthur Visit

On June 19, our trustee Wendy Russell, a member of Winterthur Museum and Gardens, helped arrange for the Lab to be part of a special Winterthur visit to Long Island's North Shore. Their schedule included Old Westbury Gardens, the Planting Fields Arboretum, the model railroad and gardens of Mr. and Mrs. Henry Harris, Jr., as well as private gardens of local garden club members. Here at the Lab they visited our DNA Learning Center, heard from lab scientists, and took a tour by car of the grounds.

Advocates for Cancer Research

Our relationship with the very dynamic grass roots organization 1 in 9: The Long Island Breast Cancer Action Coalition continues to flourish, as does Mike Wigler's research that they help support. Susan Cooper and Wendy Goldstein again attended the annual Michael Scott Barish Sand Soccer Tournament in Long Beach, where hundreds of children play soccer to support the work of 1 in 9. More than a dozen Lab staff took
part in their walk-a-thon in Eisenhower Park on May 19, at which Nassau County Executive Tom Gulotta dedicated the Garden of Hope in memory of the founding members of 1 in 9 who lost their battle with breast cancer. At their second annual black-tie dinner dance at the Seawane Club in Hewlett Harbor, Jim Watson was honored in the good company of New York State Senator Joseph L. Bruno, Nassau County Executive Thomas S. Gulotta, and proactive union leader Joseph Scalamandre. During this elegant event, 1 in 9 president Geri Barish presented the Laboratory with a $50,000 check toward the 1 in 9 grant in support of cancer research in the Wigler lab. We were honored to be there and continue to be deeply grateful for their support.

Capital Projects
Our buildings and grounds staff (the new Facilities department), housed for many years in the garage-style buildings facing the harbor on the Lab’s lower roadway, has officially outgrown its space. To that end, we have completed renovation of the former Kurahara House and begun construction of two adjacent barns approved for construction by the Village of Laurel Hollow on June 17, 1996. This new complex, located at the north end of the campus west of Bungtown Road, will consolidate all facilities, functions and staff, including painters, landscapers, security, electricians, grounds keepers, plumbers and others. The Richards Complex is named for former director of buildings and grounds Jack Richards, who continues to serve part-time as director of construction.

Bungtown Road was once again paved in 1996. While it is, in fact, a public road, the Lab has for many years maintained, plowed and otherwise cared for our “main road.” This is a benefit to the Lab and our neighbors alike.

Record Snow
The early months of 1996 were marked by the most profound snowfall in the history of the Laboratory. On the eighth day of January, the “Blizzard of 96” deposited more than 20 inches of snow into 50 mph winds, closing schools throughout the area and many local roads. While most Laboratory staff stayed home for a “snow day,” dedicated members of our Facilities Department braved the elements and traveled to the Lab to clear walks and roads on Lab grounds, and mechanics arrived to assure support for labs where research continued through the storm. On April 10, yet another foot of snow blanketed the grounds during the twentieth snow storm of the season, making the winter of ’95/’96 the snowiest on record. The Facilities staff worked tirelessly to keep the campus safe and clear, and for this we are most appreciative.

Long-Term Service
At a festive poolside celebration at Banbury Center, Bill Keen, our comptroller who has kept the Laboratory financially responsible year after year, celebrated 25 years with not a single adverse institutional audit report. Bill calmly manages the complex nature of our funding with skill and
humor. John Maroney, assistant administrative director/manager of commercial relationship, also marked a quarter century of service. John began in 1971 as a laboratory technician for James Lab, where he learned the buying skills necessary for a 1975 promotion to the Lab’s purchasing manager. In 1982 John assumed the position of assistant administrative director. In 1989 he earned his law degree, which he applies to his biotechnology licensing responsibilities.

Chief accountant Guy Cozza, groundsman Joseph Ellis, administrative assistant Roberta Salant, assistant director of facilities Peter Stahl, and media maker in Demerec Lab Margaret Wallace each celebrated 20 year milestones.

The Press’ senior technical editor Dotty Brown; James lab aide Rodney Chisum; senior scientist David Helfman, who was overseas on sabbatical for most of the year; grants specialist Pat Kurfess; carpenter Joseph Pirnak; senior photo technician Phil Renna, and editorial coordinator Liz Ritcey each celebrated 15-year anniversaries with the Lab.

The evening ended with a presentation of gifts to honor each person’s service.

Changes in Scientific Staff

Turnover in scientific staff is to be expected in a rigorous and dynamic scientific research institution such as Cold Spring Harbor Laboratory. This year, after more than two decades at Cold Spring Harbor, senior scientist Mike Mathews left us for the University of Medicine and Dentistry of New Jersey (UMDNJ) in Newark, where he accepted a position as Professor and Chairman of the Department of Biochemistry and Molecular Biology. Mike arrived here in 1974 as an early member of the “tumor virus group” and was studying diverse aspects of regulation of gene expression in recent years. I was most fortunate to work with him as a Postdoctoral Fellow when I first came to the Laboratory and I wish him well in his new position. Also, Venkatesan “Sundar” Sundaresan left after ten years of plant research with us. Sundar was instrumental in establishing the current plant genetics research program at the Lab. Together with Rob Martienssen he developed the use of Barbara McClintock’s transposons from maize to study plant development and gene expres-

Mike Mathews
(Photo by Susan Cooper)
sion in *Arabidopsis*. Sundar has gone on to become Director of the Institute of Molecular Biology at National University of Singapore.

Kim Arndt, an Associate Investigator who studied cell cycle in yeast and had been with us since 1988, left for a position as Principal Scientist with Wyeth-Ayerst Research Laboratory in Pearl River, New York. Staff Associate Akiya Watakabe left David Helfman’s lab to join the faculty of the National Institute for Basic Biology in Okazaki, Japan.

Ronald Pruzan, visiting scientist from Geron Corporation, wrapped up his stay in Carol Greider’s lab. He has returned to Geron, the biotech company in Menlo, California that is working to develop Carol’s work on telomerase into clinical applications.

**Arrivals**

We had ten new visiting scientists join our staff this year. One, Clifford Yen, did postdoctoral research here in Rich Roberts’ lab in 1978-1982 and is now looking at genetic mutations in human cancers using RDA (representational difference analysis) in Michael Wigler’s lab. Daniel Bush came for his sabbatical from USDA-ARJ and the University of Illinois to do plant research with Rob Martienssen; Al-Ping Dong, from Northeast Normal University in China, is working with X-ray crystallographer Xiaodong Cheng; Satoshi Kida came from the University of Tokyo to study learning and memory in Alcino Silva’s lab; Umberto Piarulli arrived from the Organicae Industriale in Milano, Italy, to work in combinatorial chemistry with Peter Nestler. Grigori Enikolopov has been hosting several visiting scientists—Vladimir Scheinker came from C.P.G., Inc. in Lincoln Park, New Jersey, to study the role of nitric oxide in differentiation, and Olga Zatsepina came to join her husband Boris Kuzin, who has been a visiting scientist in Grisha’s lab intermittently since 1994. Venkatesan Sundaresan had two visiting scientists studying plant genetics with him in 1996—Wei-Cai Yang from Wageningen University in the Netherlands and De Ye from Institute of Molecular Agrobiology in Singapore.

As usual, we also had a great infusion of youthful intellect. Twenty-three labs took in one or more new postdoctoral researchers this year, for a total of 33. Fourteen new graduate students also joined labs at Cold Spring Harbor.

**Promotions**

Promotions this year included plant geneticist Ulrich "Ueli" Grossniklaus, who studies plant reproductive biology and development; cancer researchers Greg Hannon, who studies cell cycle, and Scott Lowe, who studies apoptosis and cancer therapy resistance; X-ray crystallographer Ruiming Xu; and genome computer scientist Michael Zhang. Each was promoted to Assistant Investigator.

**Postdoctoral Departures**

The following postdoctoral researchers moved on during 1996. Some went on to accept new positions elsewhere, others are continuing their postdoctoral research. Four departed from David Beach’s lab; two to continue postdoctoral research—James Hudson at the Institute of Child
Health in London; and Koji Okamoto at Columbia University in New York—and two to research positions: Maureen Caligiuri as a scientist with Milotix, Inc. in Cambridge, Massachusetts and Johannes Hofmann as a staff scientist at the Basel Institute of Immunology in Switzerland. Two of Mike Mathews' researchers—Tsafrira Pe'ery and Yegnanarayana Ramanathan—accompanied him to UMDNJ while two others went on to continued postdoctoral research elsewhere: Huey-Jane Liao to SUNY Stony Brook Pathology Department and Mingsong Liu to University of California at San Francisco, Department of Medicine. Chantal Autexier and Karen Buchkovich left the Greider lab—Chantal to become a senior fellow at BioChem Pharma, Inc. in Chomedey, Canada and Karen to a Research Assistant Professorship at University of Illinois at Chicago; Michael Berg left the Stenlund lab to become a researcher at the Swedish University of Agricultural Sciences; and Hui-Zhi Cai left Ryuji Kobayashi's lab. Paul Kaufman and Rong Li departed from my lab—Paul to become a Staff Scientist for Lawrence Berkeley National Laboratory and Assistant Professor at the University of California—Berkeley and Rong to an Assistant Professorship at the University of Virginia, Department of Biochemistry in Charlottesville. Angus Wilson left the Winship Herr lab to assume an Assistant Professorship at NYU Medical Center in New York. Kim Arndt's lab saw two postdoc departures: Flavio Della Seta went on to become Maitre de Conference for the French University, Institute of Molecular Genetics in Montpellier, and May Luke is now a scientist with Berlex Biosciences in Richmond, California; Andrew Flint finished his postdoc in Nick Tonk's lab and has accepted a position as senior staff scientist at Charybdis Corporation in Bothell, Washington and with him went his wife, postdoc Catherine Flanagan of Hong Ma's. Mario Gimona and Constance Temm-Grove both left David Helfman's lab—Mario to become group leader at the Austrian Academy of Sciences, Institute of Molecular Biology and Connie to accept a position as research specialist at the University of Arizona Department of Animal Science in Tuscon. Qing Gu left the Martienssen lab to continue postdoctoral research at University of Tennessee in Knoxville; Akemi Hanamura returned to Japan from the Krainer lab; Kyoko Hidaka finished her postdoctoral work in Bruce Futcher's lab and went on to become a staff scientist at the National Cardiovascular Center Research Institute in Suita, Japan; and Yan-Fen Hu went from Nick Tonks' lab to continue postdoctoral research at the University of Virginia in Charlottesville. Nouria Hernandez's lab said good-bye to two postdoctoral researchers—Renu Mital is continuing postdoctoral studies at the Austrian Academy of Sciences, Institute of Molecular Biology in Salzburg and Frank Pessler is now a medical student at the Ph.D./M.D. program at SUNY Stony Brook. Yukiko Mizukami of Hong Ma's lab is now a visiting postdoc at the University of California at Berkeley in the Department of Plant Biology; Juhan Sedman of Arne Stenlund's lab accepted a position as a research scientist at the Estonian Biocentre in Tarfu, Estonia; Barbara Steiner left Bruce Futcher's lab to a continued postdoc at the Central Institut für Seelische Gesundheit in Ludwigshafen, Germany; Henry Tobin left the Tully lab and Marie-Luce Vignais left the Gilman lab for continued postdoctoral research at the Institute of Molecular Genetics in Montepellier, France. Wei Wen and Scott Woody also went on to additional postdoctoral research—Wei from the Wigler lab to NYU Medical
Center in New York and Scott from the Sundaresan lab to University of Wisconsin at Madison.

Graduate Students
Many of our graduate students finished their Ph.D.s and have gone on to do their postdoctoral research: from Kim Arndt's lab Andrea Doseff completed her Ph.D. and is now a postdoctoral researcher here in Yuri Lazebnik's lab and Charles DiComo went to do postdoctoral research at Columbia University in New York. Suzana Atanasoski earned her degree from University of Zurich while working in Winship Herr's lab and has returned to Switzerland. Hai Rao and Kim Gavin earned degrees while doing graduate studies in my lab—Hai went to a postdoctoral position at the California Institute of Technology in Cal Tech Division of Biology in Pasadena and Kim continues part-time postdoctoral research in my lab and is assistant editor of Genes & Development; Dezhi Liao went from the Malinow lab to become a Howard Hughes Medical Institute (HHMI) postdoctoral researcher at Johns Hopkins University in Baltimore and Deborah Taylor went from the Mathews' lab to University of Southern California School of Medicine in Los Angeles. Kathy O'Neill received her Ph.D. and went from Mike Wigler's lab to a postdoctoral fellowship in Richard Hynes' lab at MIT, and Qing-Hong Yang earned her degree in Bruce Futcher's lab and has gone on to medical school at University of California at San Francisco. Vincenzo Cestari, a graduate student on rotation in the Silva lab, is now a Ph.D. student at University of Rome in Italy.

A Busy Time Ahead
The diversity of the programs at the Laboratory is quite remarkable and I believe the high quality of our research and education is self-evident. Yet, we do not rest on past accomplishments for very long. Only five short years after a considerable expansion of the research at the Laboratory into the exciting area of learning and memory, we find that we are in desperate need of more laboratory space for this program. To take full advantage of the great successes in understanding some of the molecular underpinnings of learning and memory, it has become obvious that we should incorporate new experimental approaches. One such approach is imaging neurons and their function in an intact brain. To visualize neuronal function in live animals would be an enormous benefit, but achieving this goal will require the construction of a new research building to house an imaging center and further technical developments. Having already recruited Dr. Karel Svoboda, a physicist-turned-neurobiologist, to join our faculty in the summer of 1997 and spearhead this new direction, plans are well under way for a handsome imaging building. This project will also enable us to increase the teaching space for the courses, particularly those courses that focus on imaging of the brain. The approval of a new zoning ordinance by the Village of Laurel Hollow early in 1996 will enable careful plans for this project and others to progress.

As I look to the immediate future, there will be opportunities to change the direction of the Laboratory in addition to the expansion of our neuro-
science program. A clear opportunity has emerged from the exciting new developments in human cancer genetics, making it necessary to strengthen our research on cancer cell biology and animal physiology. Furthermore, with the modern technologies that are available, it is certain that academic institutions that focus on basic research, such as the Laboratory, should play important roles in taking the basic research to the clinic. Gone are the days when our science rattled around in the scientific literature for some time before it was "picked up" by those more clinically inclined. By judicious choice of collaborations with clinical centers and the biotechnology industry, the gap between our fundamental discoveries and human health should be very narrow indeed.

As an institution, it is imperative that we prepare for such eventualities by continuing to recruit the best young people, provide them with the appropriate facilities, and identify partners with whom we can best synergize. Each of these endeavors is very time consuming on its own, but all are well worth the effort in the long run. In short, life in and around the Laboratory will not be leisurely in the immediate future.

April 22, 1997

Bruce Stillman, Director
* Consists of Full time and Part-time Technical Support, Core Services, Publications, Meetings, Library, Public Affairs, Building and Grounds, Administrative Personnel, Banbury Center and DNA Learning Center

OPERATING EXPENSE

- Actual Expense
- Expense Adjusted for Inflation

MEETINGS & COURSE PARTICIPANTS
Newsday recently titled a lead editorial "The World Comes to Cold Spring Harbor"— and so it does, with record numbers of visiting scientists and students, and with representatives of government, the judiciary, the press, and the general public. They come to interact with the staff here and to learn from the multi-faceted research and education programs. In so doing, they help make Cold Spring Harbor what must surely be the most interesting place in the world today at the vanguard of genetics.

Last year was a particularly good time to visit and to work at the Lab. There was a remarkable flow of discovery—from new cancer genes, to the molecular basis of long-term memory, to flowering intricacies in plants. Meetings and courses on the main campus represented the cutting edge of science, and Banbury Center, the CSHL Press, and the DNA Learning Center added richness and diversity to an already overflowing mix.

It was also a satisfactory year from the administrative standpoint, with good financial results. Revenues reached a new high of $45,215,000 and a small surplus of $59,000 was achieved after allowing for approximately $3 million of depreciation. It was not necessary to utilize any portion of the $1,150,000 of designated reserves set aside in previous years to defray start-up expenses of the neuroscience program and for any research funding shortfalls. There were, however, some significant variations in several areas of Laboratory operations. The CSHL Press incurred an unanticipated deficit of $258,000, despite higher than projected sales of already published titles. The shortfall was due to the inability to meet scheduled year-end publication dates for a number of new books and lower than expected journal advertising revenues. A reorganization program to deal with these problems was instituted shortly after year-end and already shows positive indications for 1997. The deficit at the Press was more than offset by better than anticipated financial results at the main Lab, which benefited from higher investment income and indirect cost recovery from grants; and also from lower than budgeted costs in several administrative departments. Before depreciation, 1996 operations generated positive cash flow of $3,047,000, the best since $3,066,000 in 1992. Over the past 8 years, Laboratory operations have generated more than $20 million of positive cash flow, which has been invested primarily in renewing and improving infrastructure, modernizing laboratory facilities, and acquiring new scientific equipment.

Not surprisingly, it becomes more difficult each year to repeat the good financial results to which we have become accustomed. Limits on government funding for science, and intense competition for available grants, have been accompanied by reductions in government reimbursement for overhead. Categories of costs such as computer services and scientific secretarial salaries are no longer fully reimbursed and must be paid for from in-house institutional funds. Housing subsidies for young scientists seeking an acceptable living standard here on the expensive North Shore of Long Island now total nearly $170,000 per year. The Lab will be subsidizing its new on-grounds child care center, due to open in mid-1997, and will have to provide for substantial start-up costs. Although we are exempt from most federal, state, and local taxes, the Laboratory makes substantial voluntary payments to local villages in recognition of services provided, and to the local school district on behalf of the children of staff living in on-grounds tax-exempt housing. Such payments made from institutional funds last year were approximately $155,000.
To deal with such financial realities, it is imperative to maintain the lean and relatively low-cost administrative structure which for many years has characterized the Lab, while being certain that the extensive services required by a world class scientific staff continue to be provided at a commensurate level. We are making substantial investments in facility modernization, which will reduce costs in future years. A good example is the now halfway completed $1.7 million program to replace the outdated HVAC systems in Demerec Laboratory and in Bush Auditorium, and also the lighting and electric drive systems in these and many of our other buildings. Overall, this project should generate energy savings of more than $300,000 per year and pay for itself entirely in less than six years' time.

Revenue enhancement provides a good counterbalance to increasing costs and we continue to encourage David Stewart, director of Meetings and Courses, and his staff to extend the now several-year effort to increase use of our much expanded and improved meetings and teaching facilities by adding new meetings and courses appropriate to the character and mission of the Laboratory. Each year, the number of participating scientists rises, providing significant new revenues.

Very important also has been the success of technology transfer under the very capable leadership of John Maroney. In 1996 for the first time, royalty-derived income was nearly $2 million and has become a significant source of support for the science program. This is a matter of satisfaction, but paradoxically also of concern, in that one very important patent, accounting for more than half of all royalty-derived income, will expire in the year 2000. There will be new, but probably more modest, sources of royalty income by then. In addition, the Laboratory now has growing holdings of stock in biotechnology companies, received in return for granting various rights to Lab-developed intellectual property. The Laboratory now holds equity positions of varying sizes in ten such companies based in whole or in part on Lab technology. Back in 1992, the Laboratory's Board of Trustees established the Science Fund as an integral part of the Lab's endowment, into which are placed all such stock holdings to be accumulated and used eventually for the support of the science program. For the first time, in 1996, the Lab's financial statements include a conservative valuation for such holdings of $2,672,000. Five of the companies have now completed IPOs and have public markets. While in most instances the Lab's percentage ownership of each company is relatively small, it is a significant indication of the value of CSHL technology that these 10 companies together have an overall public and private market capitalization of just over $1 billion.

Nineteen ninety-six was another outstanding year for the Laboratory's endowment, which collectively includes the Robertson and Cold Spring Harbor Funds and is our primary source of internal funding for science. With the bull market of recent years continuing to drive equity markets to record levels, the year-end combined values of the Robertson Research and Cold Spring Harbor Funds approached $130 million, which when offset by the Lab's $30 million of outstanding tax-exempt debt, represents a total of nearly $100 million, an amount we could only dream about just a few years ago. As in the past, the endowment includes a balanced mix of investments in equities, fixed income securities, and short-term instruments. Total return for 1996 was a shade less than 16%, ahead of the benchmark index, which rose 14.9%. As in past years, the firm of Miller Anderson & Sherrerd, now a subsidiary of Morgan Stanley & Co., provided outstanding investment management. During 1996, considering the substantial growth of the funds, the Finance & Investment Committee of the Laboratory, headed by John Reese, conducted a search for additional investment managers. Effective July 1, the Committee appointed two, the Vanguard Prime Cap Fund and Essex Investment Management Company, Inc., to provide additional expertise in the growth equity areas of the portfolios. U.S. Trust Company continues to provide excellent management for our short-term investments. As in the
past, the annual drawdown policy for the endowment was maintained at a conservative average of less than 3.5% of market value. This policy, together with good investment management, has enabled the endowment to grow over the years at a rate sufficient to provide an offset for inflation and for expanded science programs.

Late last year, the Buildings & Grounds Department, under the leadership of Art Brings, director of facilities, initiated its happily anticipated move into the new Richards Buildings & Grounds Facility. Named to honor Jack Richards, now our still very active director of special projects, the new facility includes modern central administrative offices and a conference room fashioned from the shell of the previous residence of Mitsu Kurahara, which was purchased by the Lab in 1992. It is flanked on either side by two new barnlike buildings which will house the various trade shops and the storage facilities needed for supplies and equipment. Well hidden from nearby meadows and from Bungtown Road are the necessary areas for parking of cars and heavy equipment, for refuse processing, and for storing various grounds and building supplies. By late summer of 1997, the entire Richards facility should be complete and in use by the Department.

A second major 1996 project was the adaptive reconstruction of the de Forest Stables for use as the new Mary D. Lindsay Child Care Center. With an eventual capacity of up to 50 infants, toddlers, and preschoolers, the new Center will add significantly to the quality of life for young laboratory families. The Center will be managed by Rainbow Chimes, Inc., an experienced child care operator from Huntington, New York, who plan to utilize the most forward-looking methodologies of child care and teaching curricula.

Other important buildings and grounds projects included a change from secondary to primary metering of the Lab’s electric power, resulting in estimated cost savings of $150,000 per year, and improved reliability and redundancy of the system, thanks to the addition of a second electric feed from a second substation. Ballybung, the new President’s house, was added to the Lab’s internal electrical grid. Demerec & James laboratories, Robertson & Hooper houses, and Cabins 1-6 all received new high efficiency boilers for heat and hot water.

In Beckman Center, a new storage area was created for course equipment and new shops for machining, equipment service, and HVAC were built. Other areas of Beckman were reconfigured to provide room for a new MR facility and for installation of a new mass spectrometer.

The upstairs guest rooms, bathrooms and the central hallway of Blackford Hall were extensively renovated and redecorated, greatly improving these 90-year old overnight bachelor accommodations for meetings guests. In Grace Auditorium the former computer center office and repair shop was converted into a new full-service bookstore, the operation of which was subcontracted to Barnes & Noble College Bookstores, Inc. It is now a pleasure to see the many visiting scientists at the Lab meetings and courses congregating in the attractive and spacious new facility to view the wide variety of general science books and the latest publications of the CSHL Press. The old bookstore and copier room were converted to additional office space for Meetings and Courses. The network operating center for information services was extensively renovated to improve the efficiency and reliability of the Laboratory’s computer system. Doubleday House, severely damaged by fire early in the year, was fully restored and returned to residential use. Last but not least, the Lab’s radioactive materials license and animal care accreditation were successfully renewed on a timely basis, completing a year of accomplishment by the B&G staff, which never fails to amaze us.

Public Affairs and Development, capably directed by Susan Cooper, was also very active and successful in areas of much importance to the Laboratory. Foremost was the increased coverage of Lab research obtained in key national and local media. Newspapers such as the
Wall Street Journal, New York Times and Newsday carried prominent articles describing cancer-related discoveries in the labs of David Beach, Mike Wigler and Carol Greider, and new insights regarding the molecular basis of long-term memory from the labs of Tim Tully, Jerry Yin, and Alcino Silva. ABC, CBS, and Cablevision News 12 all ran TV coverage of ongoing in-lab research; and more recently, following the publicity surrounding the cloning of the sheep Dolly, there was a televised discussion of bioethics with Jan Witkowski, director of Banbury Center.

Much effort was also directed toward further improving relations with the local community. A new newsletter, The Lab Next Door, described the lectures, concerts, and other events of public interest offered free of charge to the community by the Lab; and also reported the extensive science educational programs and other assistance made available to local public and private schools on a voluntary basis by our staff.

Development focused primarily on the difficult triple play of asking more-or-less the same constituency of donors to participate in a capital campaign, major benefit, and the annual fund, all in a single year. The capital campaign, for the benefit of the Lindsay Child Care Center, now has pledges exceeding $800,000 and it is clear that the goal of $1 million will be exceeded—owing much to the outstanding leadership of chairman Carol Large and vice-chairman Deborah Solbert. The Emanuel Ax benefit, led by chairman Lola Grace, was both a spectacular artistic and financial success, providing $136,000 for the benefit of the Undergraduate Research Program endowment. The CSHL Association's Annual Fund, led by president John Cleary, amazed us all by raising $608,000 and nearly matching the record $662,000 total of the previous year, despite the other demands placed on its almost 700 members.

The Corporate Advisory Board of the DNA Learning Center, chaired by Jack Leahy, once again achieved a record level of support, providing $151,000, or nearly 19 percent of the DNALC's annual budget. These funds were raised through a combination of the Third Annual CSHL Golf Tournament, chaired by Horst Saalbach of Festo Corporation and held at Piping Rock Club, and by a successful Annual Fund spearheaded by the very able Laura Hundt. The DNALC has become the Lab's most important outreach program to the public and the CAB is indispensable in providing for its financial viability.

It is always with special regret that we view the departure of someone we have come to count on as much as Barbara Wang, our assistant controller, who moved to Connecticut with her family. We were fortunate indeed in having Kathy Didie of the Grants Department ready and willing to assume Barbara's responsibilities, and already we are sure that she was an excellent choice to be our new assistant controller.

Also noteworthy during 1996 were the successful negotiations conducted by our controller, Bill Keen, leading to a new three-year indirect cost rate with the Department of Health & Human Services. Although the new rate continues the gradual downtrend of recent years in the amount of overhead the Laboratory may recover on government grants, it is an acceptable rate which continues to provide urgency to our efforts to further reduce costs wherever possible. As always, we are indebted to Bill for his very able management of Lab finances.

The same may be said each year of the fine leadership provided by the heads of our other administrative departments: Susan Schultz in Grants, Cheryl Sinclair in Human Resources, and Phil Lembo in Purchasing. In addition, we are most fortunate in the very comprehensive and effective library services provided by Margaret Henderson and her staff, and for the substantial upgrading and rationalization that has been brought to Information Services by Gerry Latter and his hardworking department. Nor must we overlook the generous and efficient assistance that Roberta Salant provides each year for the needs of the Trustees and of John Maroney and myself.
Looking forward to 1997, we are particularly pleased with the much improved relations with our neighbors in the Village of Laurel Hollow. The new projects undertaken by the Lab, such as the Child Care Center and the Building & Grounds Facility, have been processed expeditiously and fairly by the Village in conformity with the new zoning ordinance. Together, we and the Village have a better understanding of each other's needs and concerns and are determined to go forward in the most constructive manner possible.

The present year will mark the transition of a number of extraordinarily productive scientists from the Lab to other institutions. Their departure and the cost of recruiting replacements will place a substantial financial burden on the Lab, and in that regard makes the balance of this year and 1998 more financially uncertain than is usually the case. While we are saddened by these departures, this sort of transition has been historically typical of the Lab and has traditionally resulted in a new influx of young scientists who often experience the most productive periods of their scientific careers while at CSHL. They will surely continue to astound us with their discoveries, which are as often unexpected as they are unpredictable.

G. Morgan Browne
Administrative Director
TUMOR VIRUSES

The Tumor Virus section uses viruses to probe cell function and to understand the processes affected during cell transformation. Two members, Arne Stenlund and Bruce Stillman, study DNA replication of papillomavirus and SV40, respectively. The Stillman laboratory complements studies of SV40 DNA replication with studies of cellular DNA replication in human and yeast cells. Winship Herr, Adrian Krainer, and Michael Mathews study control of gene expression in human cells, including the control of transcription, pre-mRNA splicing, and translation. Yuri Lazebnik and Scott Lowe (see CSHL Fellows Section) study how programmed cell death or apoptosis is regulated in normal and cancer cells, and how changes in the regulation of apoptosis influence the response of cancer cells to chemotherapy. Jacek Skowronski studies virus/host-cell interactions with human immunodeficiency virus, probing how the viral protein Nef disrupts multiple aspects of signal transduction in T cells. The vitality of this section is exemplified by the strength of the DNA tumor virus program project which covers much of the research in this section. This program project turned 25 this year and was provided renewed funding for another five years with high praise.

DNA SYNTHESIS

Our laboratory has focused on the mechanism and regulation of genome duplication in eukaryotic cells. Traditionally, we have studied the replication of simian virus 40 (SV40) and have used this system to elucidate the mechanism of DNA replication in cells. This research, however, is increasingly moving toward understanding the replication of cellular DNA, and in the last year, this shift in focus has progressed further. We now take a combined biochemical and genetic approach to understanding the replication of DNA in cells and the process of concomitant assembly of the replicating DNA into chromatin. To facilitate the biochemistry, we have concentrated on the production of recombinant vectors for producing proteins, a time-consuming yet, we believe, productive approach in the long term. In parallel, recent studies have focused on genetic studies to identify the proteins that are necessary for replication and chromatin assembly, expecting that the two complementary approaches will yield greater insight into the problems at hand. In the yeast, this has been greatly facilitated by the completion of the sequence of the entire Saccharomyces cerevisiae genome in the early part of 1996 and the ever-increasing presence of cDNA sequences in the human genome databases.

Mechanism and Control of DNA Synthesis at the Replication Fork

G. Cullmann, V. Ellison, R. Li, M. Waga, S. Waga

Loading proteins onto the DNA is one of the rate-limiting steps in the replication of DNA, and two
FIGURE 1 Subunit assembly of the human replication factor C. Shown above are the subunit interactions important for RFC complex assembly. Although all five subunits are required for DNA synthesis, a subcomplex of p36, p37, and p40 is sufficient for ATPase activity. The stability of p140 in the complex is dependent on the p38 and p40 subunits, whereas p40 requires p36 for maintenance in the complex.

proteins that perform this function after the initial unwinding of the DNA at the replication origin are the proliferating cell nuclear antigen (PCNA) and the replication factor C (RFC). These proteins have functional counterparts in bacteria and some of the bacterial phages, as well as in the more recently characterized genome of an archaeabacteria. RFC, a clamp-loading protein and DNA-dependent ATPase, loads PCNA onto the DNA so that it encircles the double helix. PCNA then functions as a docking protein for the incoming DNA polymerase δ, and, together, pol δ and PCNA function as a processive polymerase.

We have isolated all of the *S. cerevisiae* RFC subunit genes and have begun a combined biochemical and genetic analysis of their function. In addition, we have cloned the five subunits of human RFC in recombinant baculoviruses and expressed the proteins in infected insect cells. These proteins, when expressed individually or in combination, can be used for studying the function of RFC and the subunit-subunit interactions. Using these proteins, we have identified the protein-protein interactions that occur in the RFC complex (Fig. 1). The recombinant RFC is as active as the protein purified from human cells, and it has been possible to purify complexes that contain less than the five subunits and discover the role of individual proteins in RFC function. For example, the four small subunits form a complex and have DNA-activated ATPase activity, which was surprising since many have pointed out that the large RFC subunit has DNA-binding activity.

We continue to study the interaction of RFC and PCNA with the cyclin-dependent kinase (CDK) inhibitor, p21^cip1_. We have shown that p21^cip1_ blocks PCNA-dependent DNA polymerase activity and thus inhibits SV40 DNA replication in vitro. Surprisingly, p21^cip1_ does not inhibit PCNA-dependent DNA repair. The fact that p21^cip1_ binds CDK cyclins suggests a link between cell cycle control of DNA replication and proteins at the replication fork. We have demonstrated that PCNA can load onto a template DNA in an RFC-dependent manner in the presence of p21^cip1_, and interestingly, p21^cip1_ is also bound to the DNA, presumably via an interaction with PCNA. This suggests that the inhibition of DNA replication is caused by p21^cip1_ blocking an interaction with the polymerase or that it blocks polymerase function. The effect of the CDK cyclins on this reaction is being investigated, which may provide insight as to why the protein kinases form links to PCNA via p21^cip1_.

**Chromatin Assembly**

P. Kaufman, K. Shibahara, A. Verreault

The SV40 DNA replication system has yielded interesting insight into the mechanism of the replication-coupled assembly of the histones onto the newly copied DNA, a process that needs to occur to duplicate the chromosome in each cell cycle. We have demonstrated that a chromatin assembly factor called CAFI is required for the assembly of histones onto DNA, in addition to the DNA replication proteins, the four core histones H2A, H2B, H3, and H4, and other as yet uncharacterized proteins. CAFI consists of three subunits of molecular masses of 150, 60, and 48 kD. We reported last year the cloning of the genes encoding the three subunits of CAFI and the recognition that there existed a protein that was very related to the CAF1 p48 subunit. This protein, called p46, is 89% identical and 95% similar to the p48 protein, but it does not associate in vivo with the other CAFI subunits. In addition to its role as a CAFI subunit, the p48 subunit turned out to be identical to a component of another protein complex, the human histone deacetylase enzyme, as shown by Stewart Schreiber's laboratory (Harvard Medical School).

A clue to the role of p46 came from studies in Rolf Sternglanz's laboratory (SUNY, Stony Brook) and Dan Gottschling's laboratory (Fred Hutchinson Cancer Center) on the acetylation of histones. Histone acetylation has been shown to be coupled to replication-dependent assembly of histones onto the DNA during genome duplication in the S phase of the
FIGURE 2 Protein complexes containing a family of histone interacting subunits. The human p46 and p48, as well as the yeast Cac3p and Hat2p, form a small subfamily of related WD40-repeat proteins. p48 and Cac3p are part of CAF1 or the human histone deacetylase, p46 and Hat2p are subunits of the histone H4 acetyltransferase enzyme.

cell cycle and with silencing of gene transcription in various organisms, including the silent mating-type genes and genes that are located near telomeres in *S. cerevisiae*. A relative of the human p46 and p48 proteins was found to be a subunit of the yeast histone H4 B-type acetyltransferase. This enzyme acetylates two lysine residues near the amino terminus of the histone. These lysine residues are known to be important for transcriptional silencing and to be acetylated in newly assembled chromatin during DNA replication. The yeast acetyltransferase contains two subunits, Hat1p and Hat2p, the latter being a p46/p48-related protein (Fig. 2).

In previous studies, we have purified the human and *Drosophila* CAF1 proteins and shown them to have a similar subunit composition. This year, we have purified the *S. cerevisiae* CAF1 protein complex by complementation of the SV40-based DNA replication-dependent chromatin assembly reaction in vitro. The yeast CAF1 contains three subunits and the genes encoding these have been called *CAC1*, *CAC2*, and *CAC3*, encoding the p150, p60, and p48 functional homologs, respectively. The *CAC3* gene product is related in sequence to the acetyltransferase subunit Hat2p (Fig. 2).

Deletion of each of the *CAC* genes separately or in combination resulted in viable cells that had two interesting defects. They were sensitive to ultraviolet irradiation, which induces nucleotide excision repair, but not to γ-irradiation, which induces double-strand break repair. This was of interest to us because in a collaboration with Genevieve Almouzni’s laboratory in France, human CAF1 was shown to function in chromatin assembly associated with UV-induced nucleotide excision repair.

The deletion of the *CAC* genes also produced another interesting phenotype. Deletion of any of the *CAC* genes strongly reduces silencing of genes adjacent to the telomeres of chromosomes. The deletions result in an unstable epigenetic state near the telomeric DNA because some cells in the population contain a telomere-linked gene that is fully silenced, whereas others have the gene fully transcribed. Normally, in a wild-type strain, the telomeres repress transcription of a nearby gene. It is possible that the replication-coupled assembly of chromatin at the telomeres is necessary for maintaining a stable chromatin structure at the telomeres that can be inherited from one cell generation to the next. The *CAC1* gene is identical to *RLF2* (*Rap1p* localization factor-2; identified in Judith Berman’s laboratory, University of Minnesota, St. Paul), a gene required for the normal distribution of the telomere-binding Rap1p within the nucleus. Rap1p is known to be required for telomeric silencing.

Together, these data suggest that CAF1 has a role in assembly of chromatin in vivo but that the replication-coupled assembly is not essential. This must mean that there is another chromatin assembly pathway in the cell that can ensure the inheritance of chromosomes but cannot maintain epigenetically inherited states of chromatin. The identification of a family of related proteins as subunits of a chromatin assembly factor, a histone deacetylase and a histone acetyltransferase, suggests that these proteins might function as histone targeting proteins or histone chaperones. The emergence in the last year of both histone acetyltransferases and deacetylase, both those described here and others that are implicated in gene regulation, suggests that this modification of proteins will be an important regulatory mechanism for chromatin dynamics.

Cell Chromosome Replication


During the last year, we have expanded our studies on the mechanism of initiation of DNA replication in the yeast *S. cerevisiae*. In the last year, we have iso-
lated and sequenced a new gene, called *CDC45*, that is involved in the initiation of DNA replication, and we now are studying the function of Cdc45p and its interactions with other initiation proteins.

A mutant *CDC45* was originally isolated as a suppressor of a mutation in *CDC46*, a gene that encodes one of the minichromosome maintenance (MCM) proteins. There are six MCM proteins that are certain to be required for initiation of DNA replication in all eukaryotic species examined to date. *CDC45* was known to interact genetically with at least two of the six MCM genes in yeast. We therefore cloned the wild-type *CDC45* gene by complementation of the cold-sensitive mutant. The resulting gene was sequenced and was shown to encode a novel protein that had no significant similarity to other proteins in the database, except for a protein from *Ustilago* that had also been implicated in control of DNA replication in that organism. We have demonstrated using genetic methods that *CDC45* interacts not only with the MCM genes, but also with the genes encoding subunits of the origin recognition complex (ORC). ORC is the initiator protein that binds to origins of DNA replication in yeast and facilitates the initiation of chromosomal DNA replication. This work implied that Cdc45p may mediate an interaction between the MCM proteins and ORC which is bound to origins of DNA replication in the cell's chromosomes. Studies in other laboratories have demonstrated that the MCM proteins have a critical role in licensing the replication origins so that they are competent for a new round of initiation of DNA replication. This licensing occurs in late mitosis or early G1 as cells enter the new cell cycle.

To follow up on these studies, we have cloned *CDC45* into a recombinant baculovirus vector for expression in insect cells and have recently completed the expression of all six MCM proteins in baculovirus vectors so that they may be simultaneously expressed in insect cells. Using this technology, we have also demonstrated previously the expression of the six subunits of ORC and have reconstituted the functional ORC complex in baculovirus-infected cells. Using these reagents, we plan to continue our studies on the interaction of Cdc45p with ORC and the MCM proteins and study their roles in initiation of DNA replication.

Other studies have led to the identification of other proteins that interact with ORC. The four subunits of the DNA polymerase α/primase complex have been cloned into recombinant baculoviruses and a functional protein complex has been expressed in insect cells. The protein has been purified to homogeneity and shown to have both DNA polymerase and DNA primase activities, just like the native protein purified from yeast. We have shown by a variety of methods

![Figure 3](image-url)
that the polymerase α/primase complex interacts with ORC in cells and in vitro by direct protein-protein interactions. This work is of particular interest because it suggests that ORC may attract the polymerase α/primase complex to the origins of DNA replication by direct protein-protein interactions, thereby facilitating synthesis of RNA primers that would actually initiate DNA replication.

In previous studies, we have identified the CDC6 gene by suppression of ORC mutants. We have now gone on to show that the CDC6-encoded protein (Cdc6p) is a key regulator of initiation of DNA replication. Analysis of novel mutations in the CDC6 gene have demonstrated that Cdc6p controls the competency of replication complexes to initiate DNA replication. One mutant that we have isolated, cdc6-3, causes promiscuous initiation of DNA replication throughout the cell cycle so that initiation is no longer limited to once in every cell division cycle and to S phase.

Knowledge of the mechanism of initiation of DNA replication in yeast is now quite advanced (Fig. 3). The yeast studies provide a guide for our ongoing studies on the initiation of DNA replication in human cells. Three different cDNAs encoding three of the human ORC protein subunits have been identified, and we are currently studying the proteins with which they interact. In the last year, we have also identified the human cell encoded CDC6-related protein (HsCdc6p) and have shown that it is absent in non-proliferating cells but is present in the nucleus of proliferating cells. The cloning of the gene and cDNAs encoding the protein have suggested that the transcription of the gene is controlled directly by the E2F transcription factors that are in turn controlled by the retinoblastoma tumor suppressor protein. The discovery of the HsCdc6p and ORC subunits in human cells suggests that the mechanism of initiation of DNA replication may be very similar in mammalian cells and yeast.

PUBLICATIONS

In Press

Patent
The papillomaviruses infect and transform the basal epithelium in their hosts, inducing proliferation of the cells at the site of infection. The resulting tumors are in most cases benign and will usually regress after some time, but certain types of human papillomaviruses (HPVs) give rise to tumors that are prone to progress toward malignancy, especially frequently cervical carcinoma. In total, HPV DNA can now be found in biopsies from approximately 80% of all cervical carcinomas.

A key impediment to the study of papillomaviruses has been the inability to define a simple in vitro cell culture system for HPVs, largely due to the fact that these viruses normally require specialized differentiating cells that only with great difficulty can be generated in cell culture. Therefore, a bovine papillomavirus (BPV-1) has become the prototype virus for the papillomavirus group largely because a convenient cell culture system exists for this virus. In this cell culture system, viral gene expression, oncogenic transformation, and viral DNA replication can be studied. The DNA replication properties of papillomaviruses show some unique and interesting characteristics. As a part of their normal life cycle, these viruses can exist in a state of latency characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA appears to be tightly controlled and the viral DNA is stably inherited under these conditions. This system therefore provides a unique opportunity to study plasmid replication in mammalian cells.

In previous years, we have reported the development of a short-term replication assay that has enabled us to define the viral components that are required for viral DNA replication. More recently, we have directed our attention toward detailed biochemical analysis of the replication process. We are studying the biochemical properties of the two viral proteins (E1 and E2) that are required for viral DNA replication. We are also studying how these two proteins interact with the ori and with each other to generate initiation complexes. From these studies, we now have a relatively clear picture of the roles of the E1 and E2 proteins in replication. E1 has all the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. E1 can also function to unwind a supercoiled plasmid that contains the ori sequence. The E2 polypeptide, whose function has remained more elusive, appears to serve simply as a specificity factor for E1. Through physical interactions with both E1 and the ori, E2 can provide sequence specificity in the formation of the initiation complex.

Our attention has now turned toward elucidation of the precise biochemical events that precede initiation of replication at the origin of replication, i.e., binding of the initiator to the ori, the initial opening of the DNA helix, and the assembly and loading of the E1 replication helicase at the replication fork. Our studies indicate that the assembly of a preinitiation complex is a multistep process involving the ordered assembly of E1 molecules to generate different complexes with differing activities that in turn serve to recognize the ori, destabilize the double helix at the ori, and function as the replication helicase.

**Binding of the E1 and E2 Proteins to the Origin of Replication**

J. Sedman, T. Sedman, E. Gillitzer

The smallest sequence element from BPV that can direct initiation of replication in vivo (ori) is approximately 60 nucleotides long. This sequence contains three different, separable, elements. Two of these elements have known functions and constitute the binding sites for the viral E1 and E2 proteins, respectively. The third element has been termed the A+T-rich region and its function is unknown. We have developed a series of assays to study the binding of E1 and E2 to the ori sequence to determine the requirements for binding and for formation of productive replica-
tion complexes. By a combination of DNase protection, gel retardation, and interference assays, we have established that El is capable of binding to the ori in at least two different forms, either together with E2 to form an El-E2-ori complex or by itself to form an oligomeric El-ori complex. The El-E2-ori complex is formed by cooperative binding of El and E2 to ori and requires low concentrations of El. Formation of the El-ori complex requires higher concentrations of El and forms on the same ori sequence, but does not require E2 or E2-binding sites for its formation. To determine which of these complexes are important for replication in vivo, we have generated a large number of point mutations in the ori. These mutants have been tested for their ability to form the two different complexes and for replication. Interestingly, the results show very clearly that both the ability to form the El-E2-ori complex and the ability to form the El-ori complex are important for replication activity in vivo. The simplest interpretation of these results is that the assembly of a complex that is competent to initiate DNA replication is a stepwise reaction where the El-E2-ori and the El-ori complexes represent intermediates.

An important factor in elucidating the function of the two different El-containing complexes that can form on the ori is to understand the specific composition of the complexes and the stoichiometry of binding. This information is also important to determine if a precursor-product relationship exists between the two complexes. We have therefore performed molecular mass determinations of the two different El-containing ori complexes using a combination of glycerol gradient centrifugation and gel filtration using cross-linked El complexes. The results of these studies show that El in the absence of E2 binds to the ori as a trimer. The formation of the trimer is induced by the presence of the specific binding site for El and does not occur in the absence of DNA. Interestingly, this trimer of El is topologically linked to the ori DNA, indicating that El forms a ring-like structure that encircles the DNA. In the presence of E2, El binds as a monomer to the same binding site. High resolution footprinting as well as interference analysis demonstrates that this monomer of El binds in an identical position as one of the El molecules in the trimeric complex, making it likely that the El-E2-ori complex is a precursor for the trimeric El-ori complex. On the basis of these experimental results, we can draw a schematic model for binding of El in the two different forms (Fig. 1).

**E1 Forms a Hexameric Structure That Has DNA-dependent ATPase and DNA Helicase Activity**

J. Sedman

As mentioned above, the El protein has been demonstrated to have DNA helicase activity and also DNA-dependent ATPase activity. All DNA-dependent DNA helicases to date have been shown to have nucleotide hydrolysis capacity. To identify the form of El that has ATPase activity, we assembled El under conditions used for ATPase assays and sedimented the material on a glycerol gradient. When the gradient was assayed for ATPase activity, interestingly, the activity was found to sediment as a discrete peak with a relative molecular mass of approximately 400 kD, which is substantially larger than either the El-E2-ori complex or the trimeric El-ori complex that we have analyzed previously. Further analysis of this form of El using gel filtration has revealed that the relative molecular weight of this complex is consistent with a hexameric form of El. This same complex also has DNA helicase activity, indicating that hexameric El is the active replication helicase. These results, in conjunction with the two different El complexes that we have previously identified, indicate that El is capable of forming several different complexes with different replication-related biochemical activities.

![E1-E2-Ori complex](image1)

![E1-ori complex](image2)

**FIGURE 1** A schematic model for binding of El in the El-E2-ori complex and the trimeric El-ori complex. The El molecule in the El-E2-ori complex binds on one face of the DNA helix. In the trimeric El-ori complex, one El molecule is bound in the same position as in the El-E2-ori complex, whereas the two additional El molecules are bound on the two other faces of the helix to complete a structure that encircles the DNA helix.
Functional Interactions between the E1 and E2 Proteins

M. Berg, G. Chen, S. Kivimae

In most eukaryotic replicons that have been studied, binding sites for transcription factors constitute a part of the cis-acting sequences required for replication activity. In the majority of cases, including, for example, SV40, polyomavirus, and ARS elements from Saccharomyces cerevisiae, this auxiliary activity can be supplied by various transcriptional trans-activators with little apparent specificity. A similar requirement exists also for papillomavirus replicons; however, only the virus-encoded transcription factor E2 can serve as an auxiliary factor for replication. The requirement for E2 in replication of BPV extends beyond a mere requirement for E2 bound to the ori: A physical interaction with E1 is also required. This interaction can be detected as cooperative binding of the two proteins to the ori, when the respective binding sites are located in the correct position relative to each other. Because the E2 proteins are well conserved between different papillomaviruses and have a conserved overall structure, we tested E2 proteins from other papillomaviruses for interaction with BPV E1 and for DNA replication. E2 from HPV-11 failed to interact with BPV E1 in either of these assays. This observation presented us with an opportunity to map the regions of E2 that were required for this interaction by construction of chimeric BPV/HPV-11 E2 proteins. This approach has a distinctive advantage over conventional mutagenesis in that the overall structure of the protein can be maintained. We have used this procedure to generate a large number of chimeric E2 proteins. These chimeras were tested for their ability to interact physically with BPV E1 in a biochemical assay and for the ability to support replication in vivo. The results from these experiments have revealed a more complex situation than we had anticipated. We find that multiple regions both from the amino-terminal trans-activation domain of E2 and from the carboxy-terminal "hinge" and DNA-binding domain cooperate to generate a strong interaction with E1. We also find that the interaction between the E1 and E2 proteins appears to take place in a two-step process where an initial weak physical interaction between E1 and the DNA-binding domain of E2 allows a stronger, productive interaction between E1 and the activation domain of E2. Furthermore, we can also show that the differential specificity that is observed with E2 proteins from different papillomaviruses is a result partly of differences in ori structure, indicating that the formation of a productive interaction between E1 and E2 that will result in initiation of DNA replication is highly dependent on the position of the E1- and E2-binding sites relative to each other.

PUBLICATIONS


In Press


TRANSCRIPTIONAL REGULATION

W. Herr
M. Tanaka

S. Atanasoski C. Grozinger J. Reader
D. Aufero C. Hinkley P. Reilly
R. Babb C. Huang W. Tansey
M. Boutros C. Hunt-Grubbe A. Wilson
M. Cleary Y. Liu X. Zhao
R. Freiman V. Meschan

We study the mechanisms of transcriptional regulation in eukaryotes, and use viruses to probe these mechanisms. In particular, we study interactions between the herpes simplex virus (HSV) transcriptional
regulatory protein VP16 and its cellular targets. When HSV infects cells, VP16, a virion protein, is released into the infected cell, whereupon it associates with two cellular proteins—the transcription factor Oct-1 and the cell-proliferation factor HCF—on VP16-responsive elements in HSV immediate-early (IE) promoters. Formation of this VP16-induced complex results in activation of transcription of the HSV IE promoters.

Our studies continue to focus on three general areas: (1) the study of how activators such as VP16 and the basal transcriptional machinery interact to stimulate transcription, (2) the mechanisms of formation of the VP16-induced complex, and (3) the cellular functions of the VP16-associated protein HCF.

**Enhancer Function**
C. Hinkley, P. Reilly, W. Tansey, X. Zhao

Enhancers are promoter elements that can stimulate transcription even when positioned at great distances from the transcriptional start site. To study enhancer function, we examine how enhancer-binding activators stimulate transcription through interactions with the basal transcriptional machinery assembled on core promoter elements. In vivo, it is difficult to study how the basal transcriptional machinery responds to activators because the basal factors are highly conserved, ubiquitously expressed, and probably essential proteins; there is thus no direct way to study basal factor mutants within a cellular context. To circumvent these limitations, we have taken an altered-specificity approach, using genetically tagged basal factors to analyze parallel, manipulable, transcription complexes within the cell. Figure 1 demonstrates how, by linking two such altered-specificity interactions together in sequence, we can study how two basal factors, TBP and TFIIB, function in human cells.

Our approach begins with a mutation in the TATA box of a reporter gene, in which the wild-type TATA sequence is changed to TGTA. As shown in lanes 1 and 2, this mutation disrupts activity of the reporter gene. Expression of a form of the TATA-box binding protein TBP (called TBP_{AS}), which, unlike its wild-type counterpart, can bind to the "TGTA" box, suppresses this reporter mutation and restores near wild-type levels of reporter activity (lane 3). The recent solution of the TATA box–TBP–TFIIB cocrystal structure enabled us to extend our in vivo analyses to include TFIIB. Out of the many TBP–TFIIB contacts revealed in the structure, we noticed that a glutamic acid at position 284 (E284) of TBP makes a polar side-chain–side-chain interaction with an arginine at position 169 (R169) in TFIIB. We first showed that changing E284 of TBP to an arginine (TBP_{AS-E284R}) disrupts TBP function in vivo (lane 4). We then found that a TFIIB molecule with the complementary mutation—R169 changed to glutamic acid TFIIB (TFIIB_{R169E})—can suppress this defect in TBP (lane 5), restoring wild-type levels of promoter activity.

These manipulations between TBP and TFIIB establish a three-way "sequential altered-specificity" TATA–TBP–TFIIB array that now links TFIIB activity to an output from the reporter gene, allowing TFIIB mutants to be analyzed in vivo. The array has also let us probe how TBP and TFIIB function together to support transcriptional activation. Although many activation domains use the TBP–TFIIB interaction in vivo, we have found that the known TBP–TFIIB interaction is apparently not involved in response to a glutamine-rich activation domain from the transcription factor Sp1: This activation domain is relatively insensitive to the E284R mutation in

**FIGURE 1.** Sequential altered-specificity interactions permit analysis of TFIIB function in vivo. The figure shows an RNase protection analysis of internal reference and reporter transcripts after transient expression in human HeLa cells (see text for details).
TBPAs and nonresponsive to the TFIIBR169E suppressor. This surprising result suggests that transcriptional activation in vivo can be achieved through the differential reliance on interactions between core components of the basal machinery.

**Viral Trans-activation**

D. Aufiero, R. Babb, M. Cleary, C. Huang, C. Hunt-Grubbe

We study the structure and function of the VP16-induced complex and compare that complex to cellular complexes formed by Oct-1 with a basal transcription complex and a cell-specific coregulatory protein. We continue to examine how the VP16 protein of different herpesviruses is able to associate with the same Oct-1 and HCF molecules but form VP16-induced complexes of differing DNA-binding specificities. For example, the VP16 protein of bovine herpesvirus (BHV) can form a complex with human Oct-1 and HCF on BHV VP16-response elements, but this BHV VP16-induced complex does not recognize HSV VP16-response elements; in contrast, the HSV VP16 protein forms a complex on HSV VP16-response elements but not a BHV VP16-response element. By performing transcription assays in vivo, we have shown that the differing specificities of VP16-induced complex formation by BHV and HSV VP16 protein translate into differing specificities of promoter activation. These studies show how a single transcriptional regulatory protein such as Oct-1 can acquire different regulatory potentials through association with coregulators of differing DNA recognition specificities, in this case the VP16 proteins.

Oct-1 itself displays a remarkable degree of flexibility in how it recognizes cis-regulatory elements, and we continue to investigate how flexible DNA-binding specificity is achieved. The structure of the Oct-1 DNA-binding domain—a POU domain—has offered an explanation for how Oct-1 can bind to a variety of different sequences. It is a bipartite DNA-binding domain, consisting of two separate DNA-binding structures—a POU-specific (POUs) domain and POU-homeo (POUH) domain—tethered by a flexible linker. The bipartite nature of the POU domain suggested that the POU DNA-binding structures might adopt different conformations on the DNA.

To study how the two POU DNA-binding structures bind to different regulatory sites, we adapted, in collaboration with Shannon Pendergrast (Hernandez Laboratory), a protein-to-DNA cross-linking strategy developed by S. Pendergrast and R. Ebright at the Waksman Institute. They showed that a photoactivatable cross-linker could be covalently attached to a specific cysteine in a DNA-binding protein and used to cross-link the protein to DNA. The site of cross-linking could then be mapped on the DNA. We used this method to create cross-linkers that are specific for particular domains within a protein, by placing the photoactivatable cross-linker on either the POUs domain or the POUH domain. By using such domain-specific cross-linkers, we showed that the two POU DNA-binding structures do indeed position themselves differently on different binding sites.

To our surprise, we also learned that on one type of HSV IE promoter VP16-responsive element, the structure of the POU domain is dynamic, being able to adopt at least two very different conformations on a single binding site. These results raised an intriguing question: If the Oct-1 POU domain can adopt multiple conformations on a single binding site, is VP16 able to form a complex with either conformation? To answer this question, we developed a "cross-linking interference" strategy. In this experiment, we cross-linked the POU domain to the VP16-responsive site before forming a VP16-induced complex. By cross-linking the POU domain to the DNA, we effectively fixed the position of a POU DNA-binding structure to the DNA and then asked if the cross-linked species could still form a VP16-induced complex. The results show that although sensitive to cross-linking of the POU domain, VP16 is insensitive to the position to which the POUs domain is cross-linked. Thus, multiple conformations of the POU domain are able to form a VP16-induced complex.

**Cellular Functions of HCF**

M. Boutros, R. Freiman, Y. Liu, A. Wilson

We continue to study in ever-increasing detail the role of HCF in uninfected cells. HCF is a family of polypeptides, ranging in size from 110 kD to 150 kD, which results from proteolytic cleavage of a large precursor protein of approximately 300 kD at a series of centrally located 26-amino-acid repeats called the HCF repeats. Curiously, the resulting amino- and carboxy-terminal HCF fragments remain associated with one another after cleavage.
Although HCF is a collection of large polypeptides, only the amino-terminal 380 residues of HCF are required to associate with VP16 and stabilize VP16-induced complex formation. This region consists of six repeats related to repeats found in the Drosophila protein Kelch and other proteins. We refer to the repeats in HCF as the HCF_KEL repeats. In other proteins, HCF_KEL-like sequences form a propeller-like structure in which each repeat forms a four-stranded β-sheet, representing one blade of the propeller. This propeller-like structure is also found in the β-subunit of heterotrimeric G proteins and is involved in conformation-specific recognition of the α subunit of the heterotrimeric G proteins. We hypothesize that HCF may likewise associate with a specific conformation of VP16 and by doing so stabilize the VP16-induced complex.

In uninfected cells, HCF is involved in cell proliferation. T. Nishimoto and colleagues (Kyushu University) isolated a hamster cell line that has a temperature-sensitive defect in cell proliferation caused by a missense mutation in the HCF gene. The missense mutation that causes the HCF defect lies within the amino-terminal VP16-interaction domain, and we showed that this mutation affects the ability of VP16 to activate transcription and associate with HCF. These results indicate that VP16 targets a part of the cell machinery important for cell proliferation and lead us to hypothesize that VP16 mimics a cellular cofactor of HCF function involved in cell proliferation. Perhaps by mimicking such a factor, VP16 is able to gauge the cell cycle status of the infected cell and allow the virus to make an appropriate response, either growing lytically or residing latently for many years within the host.

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**A Genetic Analysis of Transcriptional Synergy in Yeast**

M. Tanaka

In the past year, my research has focused on a genetic analysis of cooperative DNA binding by activators and of synergy in transcription. I have previously reported that activator binding to a promoter is cooperative in vivo, consistent with an earlier report on in vivo binding of the yeast activator GAL4. This cooperative binding is possibly a general basis for generating a high degree of binding-site synergy in transcriptional activation; a two-site promoter shows an approximately 30-fold higher level of transcription than the single-site promoter in my system in yeast.

The low level of transcription from the single-site promoter allowed me to isolate yeast mutants in which the activity of the single-site promoter is enhanced, presumably through more effective activator binding to a single binding site. In many of the mutants I isolated, the activity of the multiple-site promoter is relatively unaffected. As a result, the binding-site synergy of transcription seen in wild-type cells is nearly lost in these mutants.

A complementation test has shown that the mutations are recessive, indicating that loss of function of a wild-type gene(s) leads to specific enhancement of the single-site promoter activity. This, in turn, suggests that the function of the wild-type gene(s) is to repress specifically the single-site promoter, probably through selective inhibition of DNA binding. Together, these results imply that negative regulation is critical in generating binding-site synergy in transcriptional control.

**PUBLICATIONS**


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RNA splicing is a required step in the expression of most eukaryotic protein-coding genes. The selection and pairing of authentic splice sites within the spliceosome occur with a very high degree of fidelity, which requires that limited and dispersed sequence information present throughout introns and exons be precisely interpreted. The expression of many cellular and viral genes occurs via alternative splicing, which involves substantial flexibility in the choice of splice sites, allowing the expression of multiple protein isoforms from individual genes. The choice of alternative splice sites is commonly regulated to alter gene expression, either tissue-specifically or in response to a developmental program or to extracellular signals.

Both constitutive and alternative splicing mechanisms involve multiple protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. Our lab has focused on the identification, purification, and molecular characterization of protein factors that are necessary for the catalysis of splicing and/or for the regulation of alternative splice site selection.

A major portion of our efforts has been devoted to the characterization of three families of RNA-binding proteins: the SR proteins, exemplified by SF2/ASF; the heterogeneous nuclear RNP (hnRNP) A/B proteins, of which the best characterized is hnRNP A1; and the more recently discovered SF7 factors. Individual SR proteins function as essential constitutive splicing factors and are also involved in constitutive or regulated enhancer-dependent splicing. In addition, they modulate alternative splicing in vivo or in vitro in a concentration-dependent manner. This activity is antagonized by hnRNP A/B proteins to modulate alternative 5′ splice site selection and by SF7 proteins to determine alternative 3′ splice site selection.

SR proteins are required for constitutive pre-mRNA splicing and also regulate alternative splice site selection in a concentration-dependent manner. They have a modular structure that consists of one or two RNA-recognition motifs (RRMs) and a carboxy-terminal RS domain. J. Cáceres transiently overexpressed epitope-tagged wild-type or domain-deletion mutants of SF2/ASF, SRp40, SC35, and SRp20 proteins in HeLa cells to study the role of the constituent domains of these SR proteins in cellular distribution, subnuclear localization, and regulation of alternative splicing (in collaboration with T. Misteli and D. Spector, CSHL, and with G. Screaton, Oxford). Indirect immunofluorescence microscopy showed that the RS domain of SF2/ASF is neither necessary nor sufficient for targeting to the nuclear speckles and that SF2/ASF contains redundant, additive nuclear localization signals in both RRMs and in the RS domain. Striking differences were observed between SF2/ASF, which has two RRMs, and two SR proteins with a single RRM (SC35 and SRp20), since in the latter proteins, the RS domain is both necessary and sufficient as a subnuclear targeting signal to the speckles. This was shown by transiently expressing tagged chimeric proteins comprising the nucleoplasmic core domain, which localizes in the cytoplasm, and the RS domain of SF2/ASF or of SRp20. The former protein localized diffusely in the nucleus, whereas the latter was found in the nuclear speckles.

Cotransfection studies using the above constructs and alternatively spliced reporter genes were carried out to determine the role of individual domains in alternative splice site selection. As expected from previous in vitro studies, the RS domain of SR proteins is not required for the concentration-dependent switch between alternative 5′ splice sites.
RRM2 of SF2/ASF has an important role in alternative splicing specificity; deletion of this domain results in a protein that although active in alternative splicing, has altered specificity in 5' splice site selection. Thus, whereas the intact protein or SF2/ASF lacking RRM1 promotes use of the adenovirus E1A 13S 5' splice site, SF2/ASF lacking RRM2 promotes use of the 12S 5' splice site. These results demonstrate the modularity of SR proteins and the importance of individual domains for their cellular localization and alternative splicing function in vivo.

A. Mayeda has also been studying the role of individual SR protein domains in enhancer-dependent splicing and in general splicing (in collaboration with S. Chandler and X.-D. Fu, University of California, San Diego, and with G. Screaton, Oxford). The substrate specificity of the SR proteins SF2/ASF and SC35 was studied using a γ-globin pre-mRNA, which can be spliced in the presence of either protein, an HIV-tat pre-mRNA, which splices with SF2/ASF but not with SC35, and an IgM pre-mRNA, which splices with SC35 but not with SF2/ASF. Initial studies using a commitment assay showed that the individual domains of SF2/ASF and SC35 behave as functional modules. Whereas the carboxy-terminal RS domains of these proteins are interchangeable, the substrate specificity of the proteins is defined by their RRMs, of which two are present in SF2/ASF and one in SC35. RRM2 of SF2/ASF cannot function alone in splicing, but it can act positively or negatively—depending on the substrate—to modify the specificity of RRM2. Therefore, multiple RRMs in SR proteins act coordinately to achieve a unique spectrum of pre-mRNA substrate specificities.

STRUCTURE AND FUNCTION OF hNRNP A/B PROTEINS
We have continued to characterize several human hnRNP A/B proteins, which A. Mayeda previously showed to function in alternative splice site selection by antagonizing SR proteins. In collaboration with S. Munroe (Marquette University), A. Mayeda...
showed that both RRMs and the carboxy-terminal glycine-rich domain of hnRNP A1 are required for alternative splicing function. Interestingly, the two RRMs, which are closely related in sequence, are not functionally equivalent. Duplication of one of the domains and deletion of the other results in a protein with weak activity. Current experiments are aimed at identifying specific residues responsible for the differences between the two RRMs. These experiments will be guided by the structure of both RRMs together, as recently determined by our collaborator R.-M. Xu here at the Laboratory.

RNA-binding studies carried out by I. Watakabe, a former technician, and by A. Hanamura, a former postdoc, showed that hnRNP A1 and A2 bind to related sequences with high affinity, recognizing a short motif within a suitable larger context. This high-affinity binding requires both RRMs of hnRNP A1, and the carboxy-terminal domain is also required for stable interaction. Sequence-specific binding correlates well with alternative splicing function; in contrast, A. Mayeda previously showed that general RNA binding is not sufficient for activity (in collaboration with S. Munroe).

IDENTIFICATION AND CHARACTERIZATION OF A HUMAN SPLICING FACTOR INVOLVED IN CATALYTIC STEP 2

D. Horowitz identified a human splicing factor required for the second step of pre-mRNA splicing. This 343-amino-acid protein, called hPrp18, is 30% identical to the yeast splicing factor Prp18, which is involved in the second step of pre-mRNA splicing in Saccharomyces cerevisiae and is a component of the U5 snRNP. In HeLa cell extracts immunodepleted of hPrp18, the second step of pre-mRNA splicing is abolished (Fig. 1). Splicing activity is restored by the addition of recombinant hPrp18, demonstrating that this factor is required for the second step. The hPrp18 protein is tightly bound to the spliceosome only during the second step of splicing. hPrp18 is required for the splicing of several pre-mRNAs, making it the first general second-step splicing factor found in humans. Splicing activity can be restored to hPrp18-depleted HeLa cell extracts by yeast Prp18, showing that important functional regions of the proteins have been conserved. However, hPrp18 cannot functionally replace yeast Prp18, either in vivo or in vitro. A 90-amino-acid region near the carboxyl terminus of hPrp18 is strongly homologous to yeast Prp18 and is also conserved in rice and nematodes. The homology identifies one region important for the function of both proteins and may define a new protein motif. In contrast to yeast Prp18, hPrp18 is not stably associated with any of the snRNPs. A 55-kD protein that cross-reacts with antibodies against hPrp18 is a constituent of the U4/U6 and U4/U6·U5 snRNP particles and has been purified for sequencing and further characterization.

PURIFICATION AND CHARACTERIZATION OF A SPLICING-COMPLEMENTING FACTOR

We are continuing to purify and characterize additional protein factors that are essential for one or both RNA cleavage-ligation reactions. M. Murray fractionated a HeLa cell nuclear extract and obtained a fraction in which a single, or a limited number of, required component(s) has been separated from known protein splicing factors and snRNPs. This fraction is required to complement cruder fractions containing the remaining essential components, thus defining a novel activity required for cleavage at the 5' splice site and lariat formation. This activity is termed SCF1 (splicing-complementing factor 1). Extensive purification of SCF1 led to a polypeptide of approximately 75 kD and several smaller polypeptides, all of which are closely related, based on microsequence (in collaboration with R. Kobayashi, CSHL). Isolation and expression of full-length SCF1 cDNAs and further characterization of the protein are in progress.

SPLICING OF PRE-mRNAs WITH NONCONSENSUS SPLICE SITES

Q. Wu has been studying the biochemistry of splicing of a very small class of introns with unique 5' and 3' splice sites that do not conform to the consensus sequences. Whereas most introns begin with GT and end with AG, the noncanonical introns begin with AT and end with AC and are therefore known as AT-AC (or AU-AC) introns. A dozen or so examples are now known, and these introns share highly conserved 5' splice site and presumptive branch site elements, in contrast to most metazoan introns, which have degenerate 5' splice site and branch site elements. Recent work by the labs of R. Padgett and J. Steitz demonstrated that AT-AC introns are spliced by a pathway that involves several minor snRNAs—U12, U4atac, U6atac, and probably U11, as well as the major snRNA U5. It appears that the AT-AC and GT-AG spliceosomes are mechanistically similar, with U11, U12, U4atac, and U6atac performing func-
tions that are closely related to those of U1, U2, U4, and U6, respectively, whereas U5, which is thought to interact primarily with exon sequences flanking the splice sites, appears to function in both pathways. Q. Wu optimized conditions for splicing the AT-AC intron of the human skeletal muscle sodium channel α-subunit pre-mRNA in HeLa cell nuclear extracts. In contrast to previous in vitro splicing experiments with a different pre-mRNA, splicing of the sodium channel intron did not require prior inactivation of one or more major snRNAs. RNase cleavage experiments showed that this reaction required U12, U4atac, and U6atac snRNAs, but not U1, U2, U4, or U6 snRNAs. Interestingly, when the pre-mRNA substrate included a few nucleotides of the downstream conventional 5' splice site, the reaction was markedly stimulated, and this stimulation was dependent on intact U1 snRNA. This observation shows that exon-definition interactions can take place between AT-AC and GT-AG spliceosomes, and similar to exon definition interactions between adjacent conventional introns, the bridging process involves U1 snRNA bound at the downstream 5' splice site.

More recently, Q. Wu has been characterizing a small subclass of AT-AC introns, whose members lack the highly conserved consensus sequences. Interestingly, several of the sodium channel genes contain both kinds of AT-AC introns, as well as conventional introns. The divergent subclass of AT-AC introns appears to splice via the conventional pathway, with the involvement of the major snRNAs. Previous mutagenic analysis of conventional introns showed that although G to A mutation at the first position and G to C mutation at the last position abolish splicing, the double mutant is active, suggesting that a non-Watson-Crick base-pairing interaction between the first and last bases may be important for splice site recognition or proofreading. The preceding experiment shows that such introns also occur naturally and appear to splice by the conventional pathway. This raises the interesting question of why AT-AC introns are so rare, and how AT-AC introns are committed to the AT-AC versus the GT-AG splicing pathways.

PUBLICATIONS


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Gene expression in human cells is controlled by many mechanisms acting at a variety of levels. The research in our laboratory explores several different systems which exemplify this diversity of regulatory processes. Some of these involve viral genes that interact with and modulate cellular pathways, impinging on processes such as cellular growth control and viral multiplication. The following paragraphs summarize the progress made in 1996 on each topic.

Regulation of PCNA

B. Lee, M. Liu, P. Wendel, M.B. Mathews

Activation of the DNA replication machinery is an important part of the process of neoplastic cellular transformation, and the regulation of human proliferating cell nuclear antigen (PCNA) expression by the multifunctional adenovirus E1A oncogene products represents an informative system for examining the molecular mechanisms underlying transformation. PCNA is a highly conserved protein and an essential component of eukaryotic DNA replication and cell cycle complexes. The adenovirus E1A 243R oncoprotein can induce expression of the PCNA promoter through a cis-acting element termed the PERE (PCNA-E1A responsive element). The PERE contains a sequence homologous to an activating transcription factor (ATF) motif, and we previously demonstrated that the transcription factor ATF-1 is a major component of PERE-protein complexes.

We have now identified an additional PERE-binding protein, the transcription factor CREB, and shown that it forms heterodimers with ATF-1 at this site. CREB, but not ATF-1, is able to mediate transactivation of a minimal PCNA-CAT reporter by E1A 243R in vivo. Further analysis revealed that the transcriptional coactivator, the CREB-binding protein CBP, also associates with PERE-related complexes. Like CREB, CBP is able to mediate a strong transactivation response to E1A 243R at the PCNA promoter. Significantly, wild-type E1A fails to transactivate the PCNA promoter through a CREB mutant protein which does not bind to CBP. Moreover, E1A mutants deficient in their ability to interact with CBP also fail to transactivate the PCNA promoter through either CREB or CBP. These findings support a model whereby E1A 243R transactivates the PCNA promoter via a CBP-CREB-PERE pathway. In more general terms, the data outline a paradigm in which E1A 243R can target and transactivate specific DNA promoter sequences.

Regulation of HIV-1 Gene Expression

T. Pe'ery, Y. Ramanathan, M. Greenberg, M.B. Mathews

The essential HIV-1 protein Tat stimulates transcription from the HIV promoter, located in the viral long terminal repeat (LTR). Tat seems to act, at least in part, at the level of transcriptional elongation to overcome premature transcriptional termination. It has been reported that Tat binds to a cellular kinase called TAK (the Tat-associated kinase) which phosphorylates the CTD (carboxy-terminal domain) of
RNA polymerase II (pol II). Since phosphorylation of CTD is known to occur during the elongation step of transcription, it appears likely that Tat functions by recruiting TAK which then phosphorylates the CTD of pol II.

We undertook the identification of TAK and the exploration of its role in HIV transactivation. Initial attempts to purify TAK employed conventional separation procedures: Fractions were assayed for their ability to phosphorylate a synthetic peptide containing three repeats of the CTD heptamer. While developing the purification protocol, we realized that TAK's chromatographic behavior resembled that of a *Drosophila* transcription factor known as P-TEFb (positive transcription elongation factor-b). Moreover, TAK and P-TEFb both display sensitivity to the inhibitor DRB. David Price and his colleagues at the University of Iowa showed recently that *Drosophila* P-TEFb possesses a CTD kinase activity that is associated with its role as a processive elongation factor. *Drosophila* P-TEFb consists of two polypeptide chains. The small subunit was cloned and found to have similarity to cyclin-dependent kinases. Its human homolog, identified in a sequence database, exhibits 70% identity to the *Drosophila* protein. Through our collaboration with the Price laboratory, we identified TAK with human P-TEFb kinase by several criteria. First, anti-P-TEFb antibodies react with chromatographic fractions containing TAK activity, and extracts depleted with antibodies to P-TEFb exhibit a dramatic reduction in the TAK activity. Furthermore, P-TEFb appears to be the only kinase that interacts with Tat. In addition, the interaction between Tat and TAK requires an intact Tat transactivation domain, and a similar requirement exists for the interaction of Tat with P-TEFb. Finally, the inhibitors DRB and H-8 block CTD phosphorylation by TAK and P-TEFb in a similar fashion. The human P-TEFb kinase appears to participate in several different complexes in human cells. We are currently examining these assemblies with a view to understanding its roles in Tat transactivation and in the uninfected cell.

**RNA Polymerase III Termination Signals**

S. Gunnery, P. Wendel, M.B. Mathews

Observations reported last year led us to reevaluate the nature of the signals required for transcriptional termination by RNA polymerase III (pol III). We found that one of two runs of T residues inserted into a chimeric gene transcribed by pol III, VA-Tat, was not recognized as a termination signal, even though it met the known requirements for such signals. Two possible explanations were considered. First, the proximity of the T stretch to the promoter element might prevent recognition since it is placed less than 30 nucleotides from the B box of the VA RNA promoter. Second, the sequence context of the T stretch might be critical, notwithstanding its close match with the optimum context for the efficient termination of 5S RNA gene transcription reported earlier.

The first possibility was eliminated by drawing the termination signal of the wild-type VA RNA gene nearer to the B box and assaying termination efficiency. Termination was unimpaired both in vivo and in vitro when the separation was reduced to as little as 14 nucleotides, leading us to consider the sequence context of the termination signal. In the *Xenopus* 5S RNA gene, a run of four or more Ts is effective if present in a GC-rich sequence context; two or more consecutive A residues within three nucleotides preceding or following the Ts are deleterious to termination. Since the T stretch in the VA-Tat mutant complies with this context yet fails to cause termination, it is possible that the sequence requirements for the two pol III genes are different. There are two conserved sequence elements upstream of the termination signal that are conserved among different VA RNA genes, GNG lying two to six nucleotides upstream of the T stretch and a pair of nucleotides adjacent to the T stretch that are most often Cs. Site-directed mutagenesis revealed that removal of Gs from the first conserved sequence element increased the efficiency of termination and that As were not inhibitory. Similarly, introduction of A residues in place of the two Cs had no effect on termination, whereas their replacement with Gs reduced the efficiency of termination dramatically. Applying these observations to the VA-Tat mutant, we showed that removal of the Gs upstream of the run of Ts improved the efficiency of termination 40–50-fold.

This study indicates that the proximity to the promoter elements does not have any effect on the efficiency of the termination signal, whereas the sequence surrounding the Ts is crucial. Evidently, the sequence context requirement for termination of transcription varies among the classes of pol III genes.
The protein kinase PKR is an important regulator of cell activity. In addition to its well-known roles in translation (mediated by phosphorylation of the protein synthesis initiation factor eIF2) and the interferon-induced antiviral response, it has been implicated in the control of cell growth, transformation and differentiation, apoptosis, and signal transduction. Last year, we identified seven sites that become autophosphorylated when the enzyme is activated in vitro by double-stranded RNA (dsRNA) and established roles for some of them in the activation of the kinase both in vivo and in vitro. Consistent with published reports that PKR can act as a tumor suppressor, we have now shown that elimination of some of the phosphorylation sites converts PKR to a form that transforms 3T3 cells. Moreover, the transformed cells are tumorigenic in nude mice.

Although regular dsRNA, presumably of viral origin, is assumed to be the enzyme’s natural activator, PKR can also be activated by highly structured (but not perfectly duplexed) RNAs such as that of the hepatitis delta virus genome. Characterization by RNase protection analysis of the binding site for PKR on this activator showed that the enzyme interacts with a region of the RNA equivalent to about 65–70 base pairs. Similar analysis of the binding site on adenovirus VA RNAI, an inhibitor of PKR activation, revealed a protected region equivalent to only about 25 base pairs. These observations provide evidence supporting a model for PKR activation that involves dimerization as a prelude to intermolecular autophosphorylation.

We are presently testing this model further and characterizing the interactions of PKR with cellular RNA activators and substrates. In addition, we have begun to address the function of a second small adenoviral transcript called VA RNAII. Although similar to VA RNAI in many respects, and transcribed from a neighboring gene, VA RNAII does not effectively block PKR activation. To address its function, we are studying two cellular proteins (other than PKR) that interact with VA RNAII. The proteins were purified, partially sequenced, and identified through database searches as a helicase and a DNA-binding protein. Examination of the nature and consequences of their interactions with VA RNAII is under way.

PUBLICATIONS


In Press


Nagai, K., S. Li, A.H.-T. Wong, A.R. Cuddihy, N.W.N. Tam, M.B. Mathews, N. Sonenberg, J. Hiscott, M.A. Wain-


## MOLECULAR MECHANISMS OF APOPTOSIS

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Our laboratory studies the molecular mechanisms of apoptosis. Apoptosis is a fundamental biological process critical for the development of organisms and for maintaining tissue homeostasis. Consequently, deregulation of apoptosis contributes to diseases such as cancer and neurodegenerative disorders. The emerging view of apoptosis is that diverse regulatory pathways activate a conserved execution machinery that carries out cell disassembly. Although this execution machinery is poorly understood, it appears that an essential component are caspases (formerly known as ICE-like proteases), a family of cysteine proteases. Caspases are activated at the onset of apoptosis and preventing this activation prevents cell death. We study which caspases are involved in apoptosis, how they are activated, and how this activation leads to cell death. Our long-term goal, however, is to determine whether the apoptotic machinery can be used to kill cancer cells selectively.

### Identification of Caspases Involved in Apoptosis

L. Faleiro (in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory)

The key observation linking caspases to apoptosis was the finding that CED-3, a protein essential for apoptosis in *Caenorhabditis elegans*, is a caspase. This finding prompted the search for the mammalian equivalent of CED-3. However, although only one caspase is known in *C. elegans*, ten caspases have been identified in humans. The diversity of human caspases raised the question of which caspases are involved in apoptosis. Of the human caspases, only interleukin-1β converting enzyme (ICE), which processes cytokines during inflammatory response, has a well-established function. To find which caspases are involved in apoptosis is critical both for an understanding of apoptosis and for developing therapeutic strategies that target this process.

We developed an approach to identify directly the pool of caspases activated in apoptotic cells. Caspases are expressed as precursors that must be proteolytically processed to become active enzymes. This processing yields three polypeptides, a prodomain, a small subunit, and a large subunit which contains the catalytic cysteine. The two subunits associate to form the active protease and both contribute to the catalytic activity. It has been known that peptide inhibitors that mimic caspase substrates and inactivate caspases by binding to the catalytic cysteine can block apoptosis. However, the identity of the caspases inhibited remained elusive since peptide inhibitors have broad specificity and can inactivate multiple caspases. We have used peptide inhibitors tagged with biotin as affinity labels and developed an approach to simultaneously detect and identify multiple labeled caspases by their mobility in two-dimensional polyacrylamide gels. This approach provides a snapshot that identifies the caspases activated in apoptotic cells, estimates their relative abundance, and indicates their posttranslational modifications.

Using tumor cells as a model, we have found that CPP32 (caspase 3) and Mch2 (caspase 6) are the major active caspases in apoptotic cells and are activated in response to distinct apoptosis-inducing stimuli and in all cell lines analyzed. Both CPP32 and Mch2 are present in apoptotic cells as multiple active species. In a given cell line, these species remained the same irrespective of the apoptotic stimulus used. However, the species of CPP32 and Mch2 detected varied among cell lines, indicating differences in caspase processing. These results pointed at CPP32 and Mch2 as two major caspases involved in apoptosis and also demonstrated that more than one...
caspase is activated in apoptotic cells. We will focus on determining how CPP32 and Mch2 are activated and what they do to bring about cell death.

The approach that we developed relies solely on the biochemical activity of caspases and does not depend on caspase primary sequence, cell type, or the signal inducing apoptosis, making this approach widely applicable for identifying active caspases involved in apoptosis.

Oncogene-dependent Apoptosis in Extracts from Drug-resistant Cells
H.O. Fearnhead [in collaboration with Scott W. Lowe, Cold Spring Harbor Laboratory]

The failure to eliminate cancer cells effectively and selectively is a major problem of cancer therapy. Nonetheless, most mammalian cells, including those that give rise to cancer, have an intrinsic machinery whose function is to carry out cell suicide. Activation of this execution machinery results in apoptosis, a comprehensive process that can quickly eliminate large numbers of cells without triggering adverse responses such as inflammation. The efficiency of such killing prompts the examination of the apoptotic execution machinery as a potential tool for killing cancer cells.

Indeed, many anticancer drugs kill cells by activating the apoptotic machinery. However, this killing is inefficient as activation is indirect. The direct effect of these drugs is cell damage, such as DNA breaks or cell cycle aberrations, which then triggers signaling pathways that activate the execution machinery and eventually leads to cell death. However, a cancer cell may fail to die not because the drug does not induce cell damage but because the information about this damage fails to reach the execution machinery of apoptosis. In principle, agents that directly activate the execution machinery should bypass alterations which prevent apoptosis and kill cells that are otherwise resistant to cancer therapy. However, how to activate the apoptotic machinery in cancer cells directly and, most importantly, selectively is not clear.

During the last year, we tested whether drug-resistant oncogenically transformed cells retain intact apoptotic machinery. To do so, we used cell-free systems of apoptosis, a model that we developed previously. In such systems, extracts from apoptotic cells induce apoptotic changes in isolated nuclei (apoptosis in vitro), whereas extracts from untreated cells do not. Like apoptosis in vivo, apoptosis in vitro requires caspase activity, which is absent in extracts from untreated cells. As an example of drug-resistant cells, we used the 293 cell line, which was derived from human kidney cells by transformation with adenovirus DNA. 293 cells are unable to activate components of the apoptotic machinery—the ICE-like proteases (caspases)—following treatment with an anticancer drug.

Remarkably, extracts from untreated 293 cells spontaneously activated caspases and induced apoptosis in a cell-free system, indicating that drug-resistant cells have not only the apoptotic machinery, but also its activator. Comparing extracts from cells with defined genetic differences, we showed that this activator is generated by the adenovirus E1A oncogene and is absent from normal cells. We provided preliminary characterization of this oncogene-generated activity (OGA) and showed that partially purified OGA activates caspases when added to extracts from untransformed cells.

Our results suggest that a basis for selective activation of caspases in cancer cells may lie in the mechanisms intrinsic to carcinogenesis. A number of oncogenes other than E1A, when expressed in cells, induce apoptosis. However, transformed cells may survive and give rise to cancer because apoptosis is suppressed, for example, by overexpression of Bcl-2, an inhibitor of apoptosis. Such suppression not only promotes the survival of cancer cells, but also confers resistance to chemotherapeutic drugs that kill by inducing apoptosis. Whether the pro-apoptotic activity of oncogenes is abolished by proteins such as Bcl-2 or whether it becomes latent was not known. Our results argue that OGA is present in transformed cells, although in a latent form. We suggest that agents that link OGA to caspases in cells would kill tumor cells otherwise resistant to conventional cancer therapy. As this killing relies on an activity generated by an oncogene, the effect of these agents should be selective for transformed cells. Purification of OGA, which is under way, should identify this activity and provide a clue as to whether it can be used to kill transformed cells.

Regulation of Caspases
A. Doseff, J. Rodriguez, Y. Xu

Although caspases are considered a central part of the apoptotic machinery, how these proteases are regu-
lated is not known. We address the regulation of caspases by determining possible posttranslational modifications of these enzymes, identifying proteins that interact with caspases, and revealing their cellular localization. A major effort was made during last year in generating tools required to utilize these approaches. We obtained a set of monoclonal antibodies to CPP32 and are in the process of producing antibodies to other caspases. Some of anti-CPP32 antibodies can efficiently immunoprecipitate CPP32 from cell extracts. We began to exploit these unique reagents for several lines of experiments: to analyze whether CPP32 undergoes posttranslational modifications other than proteolysis and to identify proteins interacting with CPP32.

To identify proteins interacting with caspases, we also continued to exploit the two-hybrid system. This approach resulted in identification of multiple polypeptides that interact with the prodomain of CPP32 but not with the catalytic subunits. The relevance of these polypeptides to the function of CPP32 in apoptosis remains to be identified.

In Press
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CELL SIGNALING IN HIV PATHOGENESIS

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AIDS is invariably associated with the depletion of the regulatory subset of T cells that express the CD4 protein on their cell surface. These CD4+ T lymphocytes are the principal infected cells in human immunodeficiency virus (HIV) infection, which suggests that viral infection may be a lethal event for these cells. Our main interest is in understanding the molecular mechanisms underlying the pathogenesis of AIDS and, in particular, in understanding the functional consequences of the interactions between viral proteins and the cellular regulatory machinery.

In the last year, our work has focused on the functions of the Nef proteins encoded by both HIV and the simian immunodeficiency virus (SIV). Evidence from the SIV/rhesus monkey animal model of human AIDS, and evidence from individuals infected with HIV-1, indicates that Nef is critical for AIDS pathogenesis since disease does not develop in individuals that harbor nef-defective viruses. The function(s) of Nef that is critical for pathogenesis in vivo is not known, and understanding these functions has been a major focus of studies in our laboratory in the past several years.

Nef is not required for the viral life cycle under laboratory conditions, and therefore its function has been difficult to study. We found previously that Nef has several effects when expressed in cultured cells. In particular, Nef modulates several aspects of signal transduction machinery in T lymphocytes. We have defined the amino acid residues important for these functions and have disrupted the individual functions of the Nef protein. These studies revealed that Nef is involved in several separate interactions with the host cell machinery. Next, we have been using the SIV-rhesus monkey model of human AIDS to determine whether these interactions are important for the function of Nef in vivo (in collaboration with Frank Kirchhoff, University of Erlangen, Germany). Finally, during the last year, we initiated experiments designed to identify and clone the cellular proteins that interact directly with Nef and mediate its effects on cellular signaling. These experiments will provide a framework with which to manipulate Nef function in vivo.

SEPARATE FUNCTIONS OF NEF DISRUPT TWO ASPECTS OF T-CELL ANTIGEN RECEPTOR FUNCTION

Two major effects on cell function have consistently been observed with natural nef alleles. One effect is to induce alterations in cellular signal transduction
and the other is to down-regulate surface expression of the CD4 molecule. The ability of Nef to alter signal transduction has been shown by a block to interleukin-2 (IL-2) gene induction in T cells, and we have recently shown that Nef blocks the CD69 gene induction that follows stimulation via the T-cell antigen receptor (TCR) in Jurkat T cells. The ability of Nef to down-regulate CD4 surface expression is likely to be important for the pathogenic process, since the CD4 molecule forms part of the viral receptor and is also a critical component of the TCR signaling machinery.

We developed a new rapid assay to measure simultaneously the effects of Nef on two aspects of the TCR machinery, CD4 expression and CD69 gene induction. In this assay, expression of Nef in Jurkat T cells results in a dramatic reduction in CD4 surface expression and also results in a block to the induction of CD69 following TCR stimulation. This assay permitted us to characterize a large number of natural nef alleles isolated from HIV-1-infected individuals and of mutant Nef proteins. We found that the abilities of Nef to alter both CD4 expression and TCR signaling are conserved with natural Nef proteins derived from HIV-1-infected individuals and from AIDS patients, and therefore these two functions are clearly important for viral replication in vivo and for pathogenesis.

Since CD4 is a component of the T-cell signaling apparatus, the two effects of Nef on the TCR could merely reflect two consequences of a single interaction. To address this possibility, we analyzed a large number of mutant Nef proteins. These studies identified mutations that selectively disrupted the effect of Nef on CD4 expression, or on TCR signaling, indicating that these two functions of Nef reflect separate interactions. We also found that the effect of Nef on TCR signaling in Jurkat T cells does not require the association of Nef with a serine kinase from the PAK family, which abundantly associates with many SIV and HIV-1 Nef proteins.

The observation that two separate functions of Nef target different aspects of TCR machinery is intriguing. It is possible that these effects reflect the redirecting of certain key components of the T-cell signal transduction machinery by Nef to enhance viral replication and/or a coordinated effort to alter the normal pattern of T-cell responses for the advantage of the virus. In particular, the modulation of TCR signaling by Nef could alter a range of T-cell responses, including functional activation and programmed cell death, both of which could have important roles in the immunodeficiency virus life cycle in vivo.

FUNCTIONAL ORGANIZATION OF THE HIV-1 NEF PROTEIN

Analysis of a large number of mutant HIV-1 Nef proteins allowed us to identify those amino acid residues in Nef required for the effects on CD4 expression and on TCR signaling and to determine that these two effects reflect separate functions of the Nef protein. Mutations that had selective effects on the ability of Nef to down-regulate CD4 expression mapped to two regions within the Nef molecule, one region in the amino-terminal third of the molecule and the other within a short region in the carboxy-terminal part of the Nef molecule (see Fig. 1). The ability of Nef to block TCR signaling was selectively abolished by mutations in the central part of Nef molecule. This part is highly conserved both among HIV-1 natural isolates and among the other primate lentiviruses. In particular, the effect of Nef on TCR signaling was abolished by mutating the elements in Nef that are important for the association of Nef with SH3 domains. We propose that Nef interferes with T-cell signaling as a result of an SH3 domain-mediated interaction.

Within the last year, the nuclear magnetic resonance (NMR) and crystal structures of HIV-1 Nef were solved. Examination of these structures showed that the amino acid residues that affected the ability of Nef to down-regulate CD4 expression mapped to the two disordered loops in the Nef structure, one in the amino-terminal portion of Nef and one in the carboxy-terminal portion of Nef. In contrast, mutations that disrupted the effect of Nef on TCR signaling mapped to the highly structured core of the Nef molecule. Our observations indicate that Nef is involved in several molecular interactions that are required for Nef to down-regulate expression of the CD4 molecule and to block TCR signaling. At least two interactions involving the disordered loops in Nef are required for the effect of Nef on CD4 expression. Additional interactions mediated by the Nef core are required for blocking TCR signaling. These observations provided a framework to assess the contribution of the individual interactions of Nef for pathogenesis and to begin to identify the cellular partners for these interactions.

HIV-1 AND SIV NEF HAVE SIMILAR FUNCTIONAL ORGANIZATION

Expression of SIV Nef in Jurkat T cells, like the expression HIV-1 Nef, induced down-regulation of CD4 expression and blocked TCR signaling. We per-
formed a similar mutagenic analysis of SIV Nef both to establish further the degree of functional conservation between HIV-1 and SIV Nef proteins and to develop mutant SIV Nef proteins that could be used for in vivo experiments. This analysis revealed that HIV-1 and SIV Nef proteins have similar functional organization. Mutations that selectively disrupted the effect of SIV Nef on CD4 expression mapped to the regions in SIV Nef that correspond to the location of the disordered domains in the HIV-1 Nef molecule (Fig. 1). Mutating residues in the central region of SIV Nef, in contrast to mutating the corresponding residues in HIV-1 Nef, had no effect on the ability of Nef to block TCR signaling. This result was surprising, since the sequences in this region are so highly conserved, and suggested that the two core domains may interact differently with their cellular targets. To investigate this interesting possibility further, we are currently mapping the sequences in SIV Nef that are required for the blocking of TCR signaling. Experiments are also in progress to assess the importance of the interactions required for CD4 down-regulation in vivo.

THE ASSOCIATION OF SIV NEF WITH P62 PAK-LIKE KINASE IS NOT REQUIRED FOR THE INDUCTION OF AIDS IN RHESUS MONKEYS

Several HIV-1 and SIV Nef proteins associate with a p62 serine-threonine kinase related to the PAK family kinases. This family of kinases is regulated by small G proteins, and they are involved in diverse cellular processes including responses to external signals and responses to stress. To address the possible link between the association of Nef with the PAK-related kinase and Nef's role in AIDS pathogenesis was of considerable interest.

In the course of structure-function analysis of the SIVmac239 Nef protein, we identified a double amino acid substitution in Nef (P104A, P107A) that selectively disrupted the association of Nef with the PAK-like kinase activity yet did not have a detectable effect on the ability of Nef to down-regulate surface CD4 expression or to block the induction of CD69 antigen in response to TCR stimulation. Prolines 104 and 107 are located in the central conserved region of the molecule, and they comprise a putative SH3 domain-binding motif. To test whether the associa-
tion of Nef with p62 is important for the induction of AIDS, we collaborated with S. Lange and F. Kirchhoff at the University of Erlangen (Germany), who constructed a variant mac239 SIV that encoded the mutant Nef protein (P104A, P107A) unable to associate with the p62 phosphoprotein.

Interestingly, infection of two rhesus monkeys with the variant SIV resulted in AIDS and rapid death in each of the infected animals. Interestingly, no reversions were observed in the majority of nef sequences isolated from different time points during infection and from lymphatic tissue at the time of death. These observations indicate that the association of SIV Nef with the p62 PAK family kinase is not important for AIDS in SIV-infected rhesus monkeys and is surprising since this interaction is conserved for both SIV and HIV Nef proteins.

**PUBLICATIONS**


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MOLECULAR GENETICS OF EUKARYOTIC CELLS

This section comprises labs that study a diverse set of cellular phenomena, including structure, signal transduction, and cancer, using mainly, but not exclusively, molecular and genetic approaches. David Spector’s group studies the structure of the nucleus and the dynamic distribution of mRNA splicing factors using electron and fluorescent microscopy. David Hellman’s lab studies the cellular cytoskeleton, especially the variety of isoforms of tropomyosins, their origins, and their specific contribution to cellular phenotypes. Linda Van Aelst’s laboratory continues the study of small GTPases such as Ras and Rac: their roles in cellular physiology and signal transduction, and the identification and dissection, by genetic means, of their protein partners. Nick Tonks and his colleagues study the role of protein phosphatases and their substrates and modulators on the signal transduction pathways that regulate growth and differentiation. Michael Wigler’s group identifies and analyzes the genes and loci that are mutated in human cancer, and studies signal transduction in humans and yeasts. Michael Hengartner’s laboratory explores the components of the programmed cell death pathway in the nematode C. elegans, and have begun to use that model genetic organism to study memory, learning, and neural cell functioning.

MAMMALIAN CELL GENETICS

M. Wigler  C. Yen  W. Wei  L. Rodgers  B. O’Conner
M. Hamaguchi  K. O’Neill  M. Riggs  T. Chen
M. Nakamura  J. Stolarov  J. Brodsky  A. Buglino
D. Esposito  H. Tu  J. Douglas  A. Solanki
R. Lucito  K. Chang  J. Troge  A. Triger
D. Dong  Y. Han

Research in the our lab is divided into three efforts. The first and oldest part is the study of Ras signal transduction pathways. Mutant Ras were the first human oncogenes discovered, and homologs of the Ras genes are found throughout eukaryotic evolution. Our original and major objective was to understand the targets of these small GTPases, but now that we have identified the main Ras target in the budding yeast Saccharomyces cerevisiae (Toda et al., Cell 40: 27 [1985]), two targets in the fission yeast Schizosaccharomyces pombe (Wang et al., Mol. Cell. Biol. 11: 3554 [1991]; Van Aelst et al., Proc. Natl. Acad. Sci. 90: 6213 [1993]; Chang et al., Cell 79: 131 [1994]), and multiple targets in mammalian cells (Van Aelst et al., Mol. Cell. Biol. 11: 3554 [1993]; Cold Spring Harbor Symp. Quant. Biol. 59: 181 [1995]; White et al., Cell 80: 533 [1995]; J. Biol. Chem. 271: 16439 [1996]) including the Raf protein kinase, our objective has been to understand how the Ras pathways are organized.

The second part is the application of genomic analysis to the discovery of mutations underlying human pathology, with a special emphasis on cancer. This section is an outgrowth of RDA (representational difference analysis) that was developed here in collaboration with Nikolai Lisitsyn (Lisitsyn et al., Science 259: 946 [1993]), now at the University of Pennsylvania School of Medicine. Several novel genetic lesions have been discovered in breast cancer, and one new tumor suppressor gene identified in collaboration with the laboratory of Ramon Parsons at Columbia University College of Physicians & Surgeons (Li et al. 1997).

We have initiated similar efforts to identify genetic lesions in infants with de novo mutations causing a variety of syndromes.

The third part is the exploration of the utility of encoded combinatorial synthesis for the discovery of biologically active molecules. This research is an outgrowth of the development of this methodology in collaboration with Clark Still, at the Department of Chemistry, Columbia University (Ohlmeyer et al., Proc. Natl. Acad. Sci. 90: 10922 [1993]). In collaboration with Peter Nestler (CSHL), formerly of the Still lab, we are determining whether we can discover branched peptidic molecules with high affinities for
specific peptides and specific regions of proteins. The longer-term objective is to find low-molecular-weight agonists and antagonists of signal transduction pathways.

**Ras Signaling**

K. O’Neill, J. Stolarov, H. Tu, K. Chang

In the budding yeast *S. cerevisiae*, Ras proteins modulate adenyl cyclase, and Ras is in turn modulated by a guanine nucleotide exchange catalyst, Cdc25 (Broek et al., *Cell* 48: 789 [1987]; Powers et al., *Mol. Cell. Biol.* 9: 390 [1989]), and proteins that accelerate Ras GTP hydrolysis, the Ira proteins (Tanaka et al., *Mol. Cell. Biol.* 9: 757 [1989]). Three clear problems remain: How are the Iras and Cdc25 modulated; what other protein(s) is modulated by Ras; and how does Ras modulate adenyl cyclase? We are focusing on the latter problem for now. In particular, we have been investigating the role of Cap, an adenyl-cyclase-associated protein (Field et al., *Cell* 61: 319 [1990]), that is required for the activation of adenyl cyclase by Ras in vivo but not, apparently, for in vitro reconstituted systems (Field et al., *Cell* 61: 319 [1990]; Fedor-Chaiken et al., *Cell* 61: 329 [1990]; Wang et al., *Mol. Cell. Biol.* 12: 4937 [1997]).

Cap is a bifunctional protein that has been fairly well conserved in evolution (Gerst et al., *Mol. Cell. Biol.* 11: 1248 [1991]; Kawamukai et al., *Mol. Cell. Biol.* 3: 167 [1992]; Gieselmann and Mann, *FEBS Lett.* 298: 149 [1992]). The carboxyl terminus is required for cytoskeletal function and binds actin, whereas the amino terminus, required for adenyl cyclase function, binds cyclase. We discovered that the amino terminus of the mammalian Cap binds talin, a central cytoskeletal component, and that the amino terminus of yeast Cap also binds a talin homolog, Sl2. We find that these interactions, and the Sl2 protein itself, are required for yeast cells to be responsive to activated Ras. We speculate that these interactions (adenyl cyclase with Cap, and Cap with Sl2) may be required to facilitate the interaction between adenyl cyclase and Ras but have not ruled out other mechanisms. (For example, we would not exclude the possibility that Ras, in its activated state, facilitates the interaction of adenyl cyclase with these components.) In any event, these interactions may serve as a mechanism by which the assembly of cytoskeletal components control the assembly of signal transduction components and may serve as a model for the anchorage-dependent control of cell proliferation, a fundamental property of normal mammalian cells that is lost upon their neoplastic transformation.

In the fission yeast *S. pombe*, Ras is required for normal sexual differentiation and cell shape (Fukui and Kaziro, *EMBO J.* 4: 687 [1985]). The target for the latter function is probably Scd1 (Chang et al., *Cell* 79: 131 [1994]) and for the former both Scd1 and the Byr2 protein kinase (Chang et al., *Cell* 79: 131 [1994]; Wang et al., *Mol. Cell. Biol.* 11: 3554 [1991]). Byr2 is a member of the *S. pombe* MAP kinase module, that conserved triad of protein kinases that is a repeating refrain in so many eukaryotic signaling pathways. Byr2 is the functional homolog of the mammalian Raf protein kinase, and its substrate is Byrl, the functional and structural homolog of mammalian MEK. We have recently been focusing on the mechanism of the regulation of Byr2, as an ideal model system for understanding the control of MAP kinase modules in general, and have made rapid progress.

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**Models for Byr2 Activation**

*FIGURE 1* We have assembled a model for the activation of Byr2. This model is consistent with our data but is neither complete nor necessarily correct. Byr2 recognizes GTP-bound Ras1, associated with membrane. In this cellular region and through this association, Byr2 encounters other components necessary for its activation, including Shk1, also found in this region through its association with Ras (Chang et al., *Cell* 79: 131 [1994]; Marcus et al., *Proc. Natl. Acad. Sci.* 92: 6180 [1995]). Upon encountering an activated form of Shk1, Byr2 assumes an open configuration. In the presence of Ste4, which is a leucine zipper protein capable of homodimerization, the open configuration of Byr2 dimerizes, leading to its autophosphorylation and activation.
Byr2 has an amino-terminal regulatory domain and a carboxy-terminal catalytic domain. The amino-terminal domain has a binding site for Ras, Ste4 (a leucine zipper protein required for sexual function), and the catalytic domain. These binding sites have now been defined, and mutants in each have been obtained. Cells containing a mutant Byr2 unable to bind Ste4 act as though the Ste4 protein is not present; cells containing a mutant Byr2 unable to bind Ras act as though Ras-deficient; and cells containing a mutant Byr2 with impaired catalytic/regulatory interaction act as though they have a constitutively activated Byr2 kinase. These studies confirm that Ras and Ste4 are required for Byr2 function and suggest that the regulatory domain acts as an inhibitor for the catalytic domain.

Using the two-hybrid system of Fields and Song (Nature 340: 245 [1989]), we have been able to demonstrate that mutations which disrupt regulatory/catalytic interaction actually open up the configuration of Byr2 so that it is able to bind its substrate Byrl. Using this as an assay for the open configuration, we have now shown that the Shkl protein kinase (Marcus et al., Proc. Natl. Acad. Sci. 92: 6180 [1995]), the S. pombe homolog of S. cerevisiae Ste20, also required for sexual differentiation, can induce this conformational change, presumably by phosphorylating Byr2. Since Ste20 bears the same genetic relationship to the S. cerevisiae sexual differentiation MAP kinase module as does Shkl in S. pombe, and these kinases are structurally conserved in evolution, this control mechanism may represent a conserved feature in the regulation of these modules. Figure 1 illustrates one possible model that incorporates our present understandings in a consistent manner.

### Genomic Analysis

C. Yen, M. Hamaguchi, M. Nakamura,
D. Esposito, R. Lucito, Y. Han

We have been applying RDA to the analysis of human breast cancer, seeking to find loci that undergo either gene copy amplification or homozygous deletion. This work has been performed in collaboration with Larry Norton at the Memorial Sloan Kettering Cancer Institute, Scott Power's laboratory at the Amplicon Corporation, the laboratory of Ramon Parsons at Columbia University, and Richard McCombie's DNA sequencing group here at the Laboratory.

A number of procedural and technical issues had to be addressed or learned in order to conduct this work properly, involving the procurement of tissue, sorting of cancers by nuclear ploidy, performing RDA on minute amounts of DNA, verification of probe status in the absence of genomic Southern blotting due to limiting material, developing the infrastructure for chromosomal mapping, screening YAC and BAC libraries, establishing banks of cell lines and xenografts, "immortalizing" DNAs through representations, exon trapping, automated sequencing, loss of heterozygosity mapping, informatics for massive data storage and retrieval, and so on. We are still in the midst of the technical developments to accompany and fully exploit the power of RDA and the enormous bank of fixed specimens that have been archived at various medical centers. What we have learned can be applied to other cancer types as well.

The easier part has been to search for amplified loci. Nearly two dozen distinct loci have been identified that become amplified in human breast cancer. In aggregate, about 95% of aneuploid tumors are amplified at at least one of these loci, and many tumors contain multiple amplified loci. Only a few of these loci contain known oncogenes, such as erb-2, c-myc and cyclin D, so the majority should contain new genes that become activated and contribute to breast cancer. Of particular interest in this regard, we have found by careful analysis that amplification near several loci, such as erb-2 and c-myc, probably constitutes multiple and distinctly separate sites of amplification, suggesting the presence of nearby genes whose amplification may be functionally linked. It is our intention to define the genes that are overexpressed from these loci to help us narrow the field of possible breast cancer oncogene targets.

We have also identified more than a half dozen loci that appear to undergo homozygous loss. Such loci are likely to contain tumor suppressor genes. Among the loci found in this way was the locus for p15 and p16. In principle, because homozygous loss tends to occur over small chromosomal regions, the finding of the suspected tumor suppressor gene is greatly facilitated. In fact, the identification of Brca-2 was facilitated by RDA, although not by us (Schutte et al., Proc. Natl. Acad. Sci. 92: 5950 [1995]). In collaboration with Ramon Parsons at Columbia, we determined that the vast majority of loci we have identified by RDA in breast cancer arc centers for loss of heterozygosity, consistent with the idea that they contain major new tumor suppressors.

One of the loci we identified and mapped to
Chromosome 10q23 was further analyzed by our collaborator Ramon Parsons, who identified a gene, now called Pten, by exon trapping (Li et al. 1997). Subsequent analysis confirmed that the coding region for this protein is frequently mutated in advanced breast, brain, and prostate cancers. Five considerations make this an especially exciting discovery.

First, the protein has homology with protein phosphatases. Since protein kinases were among the first oncogenes identified, it is highly gratifying to finally find an enzyme that removes phosphate groups from proteins as a tumor suppressor. Second, Pten has homology with cytoskeletal proteins, suggesting it may play a part in cell-cell or cell-matrix interactions. For example, it might play a part in invasiveness, metastasis, or "ancorage-independent" growth. Third, Pten appears to be involved in many cancers, and with high frequency, suggesting it has an important and common role in cancer etiology. Fourth, loss of heterozygosity at the site of Pten appears to correlate with the transition from benign to malignant growths, suggesting it plays a part in an especially critical step in neoplasia. Fifth, work from the Parson's lab indicates that hemizygous loss of Pten appears to be the cause of a rare inherited predisposition to neoplasia called Cowden's disease.

The methodology we have developed for the analysis of mutation in cancers can also be applied to disorders in humans caused by de novo mutation within the germ line. In essence, we can look for genetic alterations present in a child that are not found in either parent. A large number of rare but profound disorders have been recognized by medical geneticists and attributed to de novo mutation, but there may also be cases that undergo frequent but more subtle disorders, such as schizophrenia and autism, that have not generally been considered to arise this way. We are in the process of developing the tools to attack such problems. If we are successful, our methods may provide an alternate and perhaps more effective manner to approach certain human disease than the widely used but cumbersome and costly methods of segregation analysis.

Encoded Combinatorial Synthesis

D. Dong

Chemists can now prepare vast numbers of chemical compounds by the combinatorial method of split synthesis (Furka et al., Int. J. Pept. Prot. Res. 37: 487 [1991]; Lam et al., Nature 354: 82 [1991]). Utilizing encoding, investigators can further identify active molecules and rapidly learn their structure. The foundation for this was established in 1993 by our collaboration with Clark Still at Columbia University (Ohlmeyer et al., Proc. Natl. Acad. Sci. 90: 10922 [1993]). In collaboration with Peter Nestler here at the Laboratory, we are using this methodology to study the combining power of branched peptidic molecules. In particular, we are searching for branched peptides that are capable of recognizing linear peptides and specific peptide epitopes in proteins. If successful, such approaches should enable us to find affinity reagents for protein molecules to detect, enhance, or diminish their function. We have found some promising molecules that appear to interact with the CAAX motif of Ras and inhibit the farnesylation of Ras. These leads and ideas will be put to more rigorous testing.

PUBLICATIONS


In Press

Our research interest is the study of the signal transduction pathways involved in cell growth control and the manner in which intracellular signaling is altered in transformed cells. Our work is focused on two major topics. The first is to investigate the role of the small GTPases Ras and Rac in signal transduction. These proteins have been demonstrated to have a central role in the signal transduction pathways that mediate such diverse biological phenomena as transformation, mitogenesis, metastasis, transcriptional activation, and cytoskeletal organization. Our major objective is to uncover the molecular mechanisms by which the Ras and Rac proteins exert these effects. The second is the functional characterization of p62dok, a constitutively tyrosine-phosphorylated, Ras-GAP-associated protein detected in chronic myelogenous leukemia (CML) progenitor cells.

Ras and Rac Signaling Pathways

B. Boettner, M. McDonough, R. Packer, L. Van Aelst

The importance of Ras in tumorigenesis has been well documented. Cellular Ras genes have been found to be frequently activated by mutation in a wide variety of human cancers. Since our discovery of the serine/threonine kinase Raf as a critical downstream target of Ras required for Ras-mediated cell transformation, evidence that additional functions of Ras contribute to mammalian cell transformation has been obtained. Ras effector domain mutants, Ras(V12,G37) and Ras(V12,C40), which were defective for Raf binding and activation retained the ability to induce potent tumorigenic transformation of some strains of NIH-3T3. The nature of these Raf-independent pathways remains unclear. Interestingly, we isolated a Ras-binding protein, AF6, which is still able to interact with the Ras(V12, C40) mutant. AF6 has been previously described in a single example as a fusion partner for ALL1 in acute lymphoblastic leukemias (Prasad et al., Cancer Res. 53: 5624 [1993]). We determined that the first 160 amino acids of AF6 were sufficient for Ras binding. The carboxyl terminus of AF6 contains an unc-104-like kinesin, class-V myosin homologous domains, and a GLGF myosin homologous repeat, suggesting a role in signaling from cell-cell junctions. We are currently investigating a role of AF6 in Ras-mediated effects on transformation, mitogenesis, and morphogenesis. Furthermore, we are determining whether AF6 localizes at cell-cell contact sites. Searching protein databases with AF6 as query, we found that the Drosophila Canoe protein showed a high degree of similarity and indeed turned out to interact with activated Ras in vitro as well. Canoe has previously been described as a downstream component in Drosophila Notch signaling (Miyamoto et al. Genes Dev. 9: 612 [1995]), thus participating in regulation of adhesive cell-cell interactions for determination of various cell fates in developmental processes. Whether AF6 could be of relevance for vertebrate Notch function will be investigated.

Compelling evidence has been provided which indicates that Rac is an essential component of Ras-induced malignant transformation. Furthermore, it has been demonstrated that activated mutant forms of Rac are able to induce tumorigenic transformation of rodent fibroblasts. Rac can also confer an invasive phenotype and metastatic potential to lymphocytes and may therefore be involved in multiple aspects of cell growth control. Despite this evidence indicating a role of Rac in tumorigenesis and metastasis, little is known concerning the normal functioning of the Rac proteins. One of the first functions attributed to Rac was its involvement in the reorganization of the actin cytoskeleton induced by growth factors and constitutively active Ras(RasV12). More recently, Rac has been demonstrated to activate the transcription factors SRF and c-Jun. Although the mechanism by which Rac activates SRF is not known, the Rac-induced activation of c-Jun is mediated by the c-Jun amino-terminal kinase (JNK). Understanding the functions of Rac and Rac-interacting proteins will be essential to elucidate the role of Rac in tumorigenesis, invasiveness, and metastasis.

Our major objective is to dissect the physiological roles of Rac and identify its interacting proteins and to further characterize the proteins that mediate the activity of Rac. Using the yeast two-hybrid system,
Functional Characterization of p62dok

N. Carpino, M. Marin, L. Van Aelst
[In collaboration with R. Kobayashi, Cold Spring Harbor Laboratory, and B. Clarkson, Memorial Sloan-Kettering Institute, New York]

The presence of a constitutively tyrosine phosphorylated, RasGAP-associated 62-kD protein (p62) was detected within chronic myelogenous leukemia (CML) progenitor cells. CML is a clonal disorder of the hematopoietic stem cell, characterized by the Philadelphia chromosome, in which exons of the ABL proto-oncogene become linked to the BCR gene on chromosome 22. The result is a chimeric protein termed p210bcr-abl, with deregulated tyrosine kinase activity. Recently, N. Carpino et al. purified and cloned the cDNA of p62, which they designated p62dok (Carpino et al., Cell 82: 197 [1997]). p62dok is a novel protein and has the features of a signaling molecule.

We have observed that p62dok becomes rapidly tyrosine-phosphorylated in hematopoietic cells in response to c-Kit ligand, in fibroblasts in response to insulin, and in v-src-transformed cells, implying a general role for it in signal transduction pathways involved in cell growth control. Our major objective is to elucidate the functional role of p62dok. We have initiated studies to determine the kinase(s) responsible for p62 tyrosine phosphorylation in normal, untransformed cells. With regards to CML, we have evidence that p62dok is a direct substrate of p210bcr-abl. In collaboration with R. Kobayashi, we are presently identifying the in vivo sites of tyrosine phosphorylation of p62dok by BCR-ABL, as well as the sites of tyrosine phosphorylation on p62dok following c-Kit ligand and insulin stimulation. The association of p62dok with Ras GAP is suggestive for a role of p62dok in the Ras signal transduction pathway, possibly by affecting the function of GAP. To address this, we are currently measuring the catalytic activity of GAP toward Ras and the GTP/GDP ratio of Ras in the presence of unphosphorylated and tyrosine-phosphorylated p62. Furthermore, we are setting up conditions to investigate a role for p62 in Ras and tyrosine-kinase-mediated transformation and are screening for p62-interacting proteins.

PUBLICATIONS


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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

N.K. Tonks
A.M. Bennett
M.A. Daddario
R.L. Del Vecchio
A.J. Garton
M.J. Gutch
K.R. LaMontagne
M.P. Myers
S.N. Mamajiwala
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The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes. Our lab is particularly interested in the role of tyrosine phosphorylation in transducing an extracellular signal into an intracellular response, such as proliferation or differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects not only the activity of the kinases that phosphorylate it, but also the competing action of protein phosphatases that catalyze the dephosphorylation reaction.

We study the expanding family of protein tyrosine phosphatases (PTPs) that, like the kinases, comprise both transmembrane receptor-linked forms and non-transmembrane cytosolic species and represent a major family of signaling enzymes. The structures of the PTPs indicate important roles in the control of key cellular functions. We are utilizing a variety of strategies to characterize the normal physiological function of several members of the PTP family.

During the last year, Andrew Flint and Yan Fen Hu completed their postdoctoral studies and took up positions at Charybdis Corporation and the University of Virginia, respectively. We were joined by Mike Myers as a postdoctoral fellow.

Identification of PTP Substrates
A.J. Flint, A.J. Garton, K.R. LaMontagne, M.P. Myers, T. Tiganis

The identification of substrates of PTPs is an essential step toward a complete understanding of the physiological function of members of this enzyme family. In 1995, in collaboration with David Barford (Oxford University), we determined the crystal structure of PTP1B on a complex with a phosphotyrosyl peptide substrate. From this structure, we defined several residues in the enzyme that were important for substrate recognition and catalysis. We have now characterized the function of these residues further by site-directed mutagenesis. Through this approach, we have converted an extremely active enzyme into a "substrate trap." Furthermore, the residue that is mutated to generate the substrate-trapping mutant is the invariant catalytic acid (Asp-181 in PTP1B) that is conserved in all members of the PTP family. Therefore, this has afforded us a unique approach to identification of physiological substrates of PTPs in general. Following expression, the mutant PTP binds to its physiological substrates in the cell but, because it is unable to
lin-15(n765ts) X  
lin-15(n765) X; cshp(op194) II

**FIGURE 1** The *C. elegans* multivulva phenotype conferred by *lin-15* loss-of-function (lf) mutation is suppressed by *cshp*(op194). LIN-15 normally functions as a negative regulator of LET-23, an EGF RTK receptor tyrosine kinase homolog. *lin-15*(lf), mutations confer a multivulva phenotype due to enhanced LET-23 activity during vulval development (left). A loss-of-function mutation in the SH2 domain-containing tyrosine phosphatase, *cshp* (op194), suppresses the *lin-15*(lf) multivulva phenotype (right), providing genetic evidence that cSHP participates in regulating LET-23 initiated signals during vulval development. (Closed arrows) pseudovulvae; (open arrows) position of the functional vulva.

dephosphorylate the target, the mutant and substrate become locked in a stable, "dead-end" complex. This complex can be isolated by immunoprecipitation and associated proteins identified by immunoblotting with antibodies to phosphotyrosine (pTyr). In addition, immunoblotting lysates of cells expressing the mutant PTP with antibodies to pTyr can be used to identify proteins whose phosphorylation state is altered as a consequence of expression of the mutant.

We have now initiated such an approach to substrate isolation with intriguing results.

**SUBSTRATE SPECIFICITY OF PTP1B**

When expressed in COS cells, the substrate trapping mutant of PTP1B forms a complex with four proteins from the spectrum of phosphotyrosyl proteins. One of these is the receptor for epidermal growth factor (EGF), which is one of the family of ligand-activated receptor PTKs. One of the others, p70, appears to be a substrate for the Src PTK, since its phosphorylation state is enhanced in cells expressing the v-Src oncogenic PTK. As would be expected for an interaction between a PTP and its substrates, the association is disrupted by the active site-directed inhibitor vanadate, and for the EGF receptor, the interaction absolutely requires receptor autophosphorylation.

Furthermore, from immunofluorescence studies, the D181A mutant of PTP1B, which is targeted to the endoplasmic reticulum (ER), appeared to retain the endogenous EGF receptor in an intracellular complex, suggesting that one important function of PTP1B may be to prevent the inappropriate, ligand-independent activation of newly synthesized EGF receptors in the ER.

We have also extended our analysis of PTP1B function in the context of the human disease chronic myelogenous leukemia (CML). CML is a clonal disorder of the hematopoietic stem cell characterized by the Philadelphia chromosome (Ph) in which the c-Abl proto-oncogene on chromosome 9, encoding a PTK, becomes linked to the *bcr* gene on chromosome 22. This results in the production of a fusion protein termed p210 bcr-abl, the PTK activity of which is enhanced relative to c-Abl. Expression of p210 bcr-abl produces the abnormal patterns of tyrosine phosphorylation that result in the dysfunctional maturation of the hematopoietic stem cell that is the characteristic of CML. Therefore, CML represents one of the best defined examples in which aberrant tyrosine phosphorylation is the underlying cause of a human disease. In contrast to the extensive characterization of the PTK and its effects on cell function, relatively
little is known about the nature of the PTPs that may modulate p210 bcr-abl-induced signaling. We have demonstrated that in cells expressing p210 bcr-abl, including a cell line derived from a patient with CML, the expression of PTP1B is enhanced specifically severalfold. The effect on expression of PTP1B requires the kinase activity of p210 bcr-abl and occurs rapidly, concomitant with maximal activation of a temperature-sensitive mutant of the PTK, suggesting that this may reflect a compensatory change to the presence of the oncoprotein PTK rather than a long-term adaptive response of the cell. Specificity in this response is illustrated by the fact that although the levels of PTP1B were increased following expression of p210 bcr-abl, we did not detect an increase in several other PTPs including TC-PTP, the most closely related homolog of PTP1B. Furthermore, specificity in the response was also evident from the perspective of the PTK in that there was no change in PTP1B levels in Rat-1 fibroblasts expressing v-Abl, which shares the same catalytic domain as p210 bcr-abl, or transformed by v-Myc, illustrating that the alteration in the levels of the phosphatase was not a general response to cellular transformation.

Our data suggest that PTP1B may function in a cellular context as a regulator of p210 bcr-abl-induced signaling events. It is known that p210 bcr-abl autophosphorylates on Y177 and that this serves as a docking site for the adaptor protein Grb2 and functions to assemble the multiprotein complex that triggers the Ras-MAP kinase cascade, a step that has been implicated strongly in transformation. Using an AP-1/Ets reporter construct to measure the activation of the Ras/MAP kinase cascade by p210 bcr-abl in a cellular context, we have shown that PTP1B was capable of antagonizing p210 bcr-abl-induced signaling. Specificity in the response is reflected in the fact that PTP1B did not inhibit transcriptional activation of the same reporter by v-Abl, suggesting that Y177 in p210 bcr-abl is a major target for the PTP. Furthermore, expression of TC-PTP or PTP-PEST did not effect p210 bcr-abl-induced signaling, as measured by this transcriptional activation assay.

Signaling by p210 bcr-abl was also inhibited by a "substrate-trapping" mutant of PTP1B, PTP1B-D181A, suggesting that a critical substrate in the signaling pathway was being held in a complex with this mutant and being rendered nonfunctional. As observed for the active PTPs, this mutant of PTP1B did not affect induction of the reporter gene by v-Abl, and an equivalent mutant of PTP-PEST did not inhibit signaling by p210 bcr-abl. Upon coexpression in COS cells, PTP1B-D181A formed a stable complex with p210 bcr-abl but not v-Abl, despite the fact that the latter was expressed abundantly and was tyrosine-phosphorylated. Furthermore, we have shown that the interaction between p210 bcr-abl and PTP1B-D181A blocked by more than 90% the association of the PTK with Grb2, suggesting that Y177 in p210 bcr-abl is a major target of PTP1B. Disruption of the interaction with Grb2 is the most likely mechanism of inhibition of p210 bcr-abl-induced activation of an AP-1/Ets reporter by wild type and substrate-trapping mutant forms of PTP1B. Therefore, the selectivity observed in the up-regulation of PTP1B levels in response to expression of p210 bcr-abl is reflected in the selectivity of PTP1B in antagonizing p210 bcr-abl-induced signaling. These data are consistent with a function of PTP1B as an antagonist of p210 bcr-abl signaling in vivo, and we are currently testing whether the effects of the PTP are sufficient to abrogate the transforming potential of this oncoprotein PTK.

IDENTIFICATION OF SUBSTRATES FOR PTP-PEST

PTP-PEST is a ubiquitously expressed, cytosolic, mammalian PTP which exhibits high specific activity in vitro. As such, one might anticipate that PTP-PEST could exert a considerable influence on the tyrosine phosphorylation status of a wide variety of proteins in vivo. We initially identified a prominent 130-kD tyrosine-phosphorylated protein in pervanadate-treated HeLa cell lysates which was preferentially dephosphorylated by PTP-PEST in vitro. We observed that substrate-trapping forms of PTP-PEST associated in stable complexes exclusively with the same 130-kD protein, which was identified as p130cas by immunoblotting. Tyrosine-phosphorylated p130cas is a signaling intermediate implicated in a wide variety of cellular processes, including oncogenic transformation, B-cell activation, and mitogen- and cell-adhesion-induced signaling; PTP-PEST is therefore potentially involved in the regulation of these processes through the specific dephosphorylation of p130cas.

We have also begun to investigate the molecular mechanisms underlying the specificity of the interaction between PTP-PEST and p130cas and have identified a high-affinity interaction between the SH3 domain of p130cas and a proline-rich sequence (P335PPKPPRT) within the carboxy-terminal segment of PTP-PEST. Mutation of Pro-337 within this sequence to alanine significantly impairs the ability
of PTP-PEST to recognize tyrosine-phosphorylated p130cas as a substrate, without qualitatively affecting the selectivity of the interaction. Thus, the highly specific nature of the interaction between PTP-PEST and p130cas appears to result from a combination of two distinct substrate recognition mechanisms: the catalytic domain of PTP-PEST contributes specificity to the interaction with p130cas, whereas the SH3 domain-mediated association of p130cas and PTP-PEST dramatically increases the affinity of the interaction. The involvement of the noncatalytic segment of PTP-PEST in the selection of p130cas as a substrate is a novel example of an emerging paradigm in tyrosine-phosphorylation-dependent signaling processes, in that substrate specificity of both protein tyrosine kinases and phosphatases is frequently influenced by the noncatalytic segments of these enzymes, offering a versatile mechanism for restricting each enzyme to specific roles in tyrosine-phosphorylation-dependent signaling pathways.

THE EGF RECEPTOR AND SHC ARE SUBSTRATES FOR TCPTP

The substrate-trapping mutants have also been used to compare the specificity of PTP1B with its closest homolog, TCPTP, in a cellular context. Alternative splicing of the TCPTP mRNA generates two forms of the enzyme that differ at their extreme carboxyl termini but share the same catalytic domain that is located at the amino terminus of the molecule. One of these forms, 48-kD TCPTP, is targeted to the ER and, like PTP1B, trapped the EGF receptor. Immuno-fluorescence studies revealed that expression of the D182A mutant of TCPTP caused an accumulation of EGF receptor in the ER. However, it did not trap the lower Mr substrates of PTP1B, particularly a prominent pTyr protein of 60 kD. The second form of TCPTP, of Mr 45,000, localizes to the nucleus. When cells expressing D182A 45k TCPTP are treated with EGF, the phosphatase exits the nucleus and can be isolated in a complex with the EGF receptor. However, unlike 48k TCPTP, this form of TCPTP also traps Shc and additional pTyr proteins of 64k and 50k. Interestingly, in this case, the EGF receptor did not accumulate in the ER, but expression of D182A 45k TCPTP appeared to block endocytosis of the receptor. The effects on signaling are currently being ascertained. These data illustrate that even closely related PTPs exhibit substrate specificity in a cellular context and suggest that control of subcellular location may be a critical element in determining such specificity.

Identification of Regulators of PTP Function

R.L. Del Vecchio, S.N. Mamajiwalla, A.A. Samatar, T. Tiganis and S.H. Zhang

In general, the structure of PTPs can be described in terms of a conserved catalytic domain to which is fused on either the amino- or carboxy-terminal side a noncatalytic segment that serves a regulatory function and can be used to distinguish individual PTPs. It is now apparent that PTPs are regulated at multiple levels. We have been characterizing a number of protein-protein interactions involving members of the PTP family that may be important in regulating activity directly or indirectly through control of subcellular distribution.

REGULATION OF CYTOPLASMIC PTPs

By affinity chromatography, using GST-TCPTP fusion proteins, we have isolated three cytoplasmic proteins of 120 kD, 116 kD, and 97 kD that interact with TCPTP. The p120 protein associated with residues 377–415 from the carboxyl terminus of the 48-kD form of TCPTP, whereas the recognition site for p97 and p116 was mapped to residues encompassing the TCPTP nuclear localization sequence (NLS). The TCPTP NLS was shown to be bipartite requiring two clusters of basic residues for efficient nuclear translocation. p116 and p97 associated with residues within basic clusters I and II, indicating a direct correlation between binding and nuclear localization. Sequence analysis of p97 identified it as the nuclear import factor p97 (importin β) which is an essential component of the nuclear import machinery. We found that TCPTP coimmunoprecipitated with the nuclear import factor p97 from cell lysates and that purified recombinant p97 and TCPTP interacted directly in vitro. In a direct comparison, the TCPTP NLS precipitated p97 and p116 from cell lysates, whereas the SV40 T-antigen and nucleoplasmin NLS did not. These results suggest that TCPTP may be targeted to the nucleus by a distinct process which may involve the direct interaction of p97 and possibly p116 with the basic clusters of its bipartite NLS. Moreover, these results demonstrate that the carboxy-terminal segment of TCPTP contains docking sites for interaction with proteins that may function to target the enzyme to defined intracellular locations and in the process regulate TCPTP function.
A second example involves PTPH1, a human PTP that has homology with the band-4.1 superfamily of cytoskeletal-associated proteins. We have found that PTPH1 interacts with the protein 14-3-3 in vivo. 14-3-3s are a family of highly conserved acidic proteins of 30 kD that are involved in control of mitogenic signaling, cell cycle, and transformation and have been shown to interact with a number of important signaling proteins. As observed for other interactions involving 14-3-3, the association with PTPH1 is phosphorylation-dependent. We have identified two novel serine phosphorylation sites in PTPH1 that serve as the major 14-3-3-binding-sites but are distinct from the consensus binding motif identified to date. Our results raise the possibility that 14-3-3 may function as an adaptor molecule in the regulation of PTPH1 and may provide a link between Ser/Thr and tyrosine-phosphorylation-dependent signaling pathways.

RECEPTOR PTPs AND CELL CONTACT

We have been characterizing PTPµ, a receptor PTP with structural similarity to the immunoglobulin superfamily of cell adhesion molecules. Our studies have illustrated that PTPµ interacts with the cadherin/catenin cell adhesion complex at adherens junctions in vivo. This interaction appears to be dynamic; conditions that lead to tyrosine phosphorylation of the cadherin/catenin complex correlate with disruption of the interaction with PTPµ. This observation is provocative because tyrosine phosphorylation of the cadherin/catenin complex has been observed under a variety of physiological conditions potentially contributing to transformation and metastasis. It appears that PTPµ may provide the regulatory balance to this phosphorylation event, thus maintaining the cadherin/catenin complex in its functional, dephosphorylated state.

A second receptor PTP that has been linked to cell contact phenomena is DEP-1 (density enhanced PTP-1), the surface expression of which is increased as cells in culture approach confluence. Recent studies have shown that expression of DEP-1 in 293 cells induced cell cycle arrest in G1 and profound cytoskeletal changes that culminated in the cells rounding up and floating off the dish. This effect, which is specific for DEP-1, suggests that the PTP may act upon cytoskeletal targets, and we are currently trying to identify and characterize these substrates.

Dual Specificity Phosphatases and Growth Control

A. Bennett

We are focusing on MKP-1, a highly selective phosphatase that dephosphorylates and inactivates members of the MAP kinase family of enzymes in vivo. The MAP kinases have been implicated as common and essential components of signaling pathways induced by diverse stimuli, suggesting that MKP-1 will be a crucial, central player in the control of cell function. We have been using MKP-1 as a molecular probe with which to examine physiological functions of the MAP kinases. Skeletal muscle differentiation is a coordinated process initiated by the activation of muscle-specific transcription factors, accompanied by irreversible cell cycle withdrawal. The myoblast cell line, C2C12, proliferates in high serum but differentiates into multinucleated myotubes upon mitogen deprivation. We have investigated whether the decision between either proliferation or differentiation in C2C12 myoblasts is determined by the level of MAP kinase activity. Within 24 hours following serum deprivation, MyoD, the muscle-specific transcription factor, was induced in C2C12 myoblasts concomitant with the down-regulation of Erk2 (MAP kinase) activity. To determine whether inhibition of MAP kinase activity by MKP-1 in C2C12 myoblasts is sufficient to induce MyoD, we generated stable C2C12 myoblast lines expressing MKP-1 (C2MKP1) under a tetracycline-repressible promoter. C2MKP1 myoblasts overexpressed MKP-1 by up to threefold upon removal of tetracycline, resulting in the inhibition of Erk2 kinase. Under conditions of high serum where C2MKP1 myoblasts fail to express either MyoD or myosin heavy chain (MHC), MKP-1 overexpression caused the up-regulation of MyoD and MHC expression. Myotube formation, however, was not observed. Thus, inhibition of MAP kinase activity by MKP-1 is sufficient to initiate the biochemical, but not the morphological, myogenic phenotype. These results also suggest that the initiation of C2C12 myogenesis may be determined by the coordinate actions of MAP kinase and MKP-1.

A Genetic Analysis of PTP Function

M. Gutch

We are using the nematode Caenorhabditis elegans as a model system for a genetic analysis of PTP func-
tion. The experiments are being performed in collaboration with Michael Hengartner's lab here at Cold Spring Harbor. An attractive feature of C. elegans is that many of the properties of its signaling pathways are conserved with those found in mammals. Thus, the lessons learned in C. elegans should be applicable to humans. Our strategy has been first to use reverse genetics, using the C. elegans endogenous transposon Tc1 to create gene knock-outs and thereby PTP-deficient nematodes. One C. elegans PTP we are focusing on is a homolog of the mammalian SHPs. This C. elegans PTP contains two SH2 domains at the amino terminus followed by a PTP catalytic domain and a short carboxy-terminal tail. Disruption of this gene by the transposon Tc1 resulted in a completely penetrant, zygotic semi-sterile/maternal-effect-lethal phenotype with abnormal oocyte maturation. A second PTP we targeted for disruption is a transmembrane PTP containing extracellular FNIII repeats and two intracellular PTP catalytic domains. A BLAST search revealed highest sequence similarity to the mammalian transmembrane PTP LAR. Targeted disruption of this gene resulted in embryonic lethality and morphologically abnormal nematodes. Future experiments will utilize classical forward genetics to identify enhancers/suppressors of the PTP knock-out phenotype.

PUBLICATIONS

In Press

THE CYTOSKELETON IN NORMAL AND TRANSFORMED CELLS

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C. Chen
M. Gimona
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M. Meyer
A. Rai

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The research in our lab is focused on the expression, structure, and function of cytoskeletal components in normal and transformed cells. One interest of our lab is the relationship between the cytoskeleton, malignant transformation, and tumor suppression. We have also been interested in understanding how specific actin assemblies are organized and regulated. How the assembly of cytoskeletal structures is regulated by extracellular signals via molecules such as rho, rac, and cdc42 and how it is altered by transformation are under investigation. Why different cell types express a specific set of structural components is still not well understood. One possibility is that the dynamic and stable macromolecular assemblies that are characteristic of a specific cell type, e.g., stress fibers, contractile ring, and filopodia in fibroblasts or sarcomeres in skeletal muscle, will require specific isoforms of given structural proteins as well as regula-
tory proteins for their proper maintenance and regulation. In some cases, isoform diversity arises via alternative RNA splicing. We are investigating the cellular function of the cytoskeletal protein isoform diversity generated by alternative RNA splicing. To study the organization and regulation of various actin containing structures, we are using a number of experimental systems derived from fibroblasts, neurons, epithelial cells, and muscle cells. In a related series of experiments, we are studying the mechanisms responsible for tissue-specific and developmentally regulated patterns of alternative RNA splicing.

During the year, three members of our group moved on to new positions. Mirjam Meyer finished her Diploma studies and has started her doctoral work at the Max Planck Institute in Berlin, Germany. After 2 years of postdoctoral study, Mario Gimona returned to the Institute of Molecular Biology, Salzburg, Austria. Akiya Watakabe has taken a staff position at the National Institute for Basic Biology, Okazaki, Japan. During most of 1996, I was on sabbatical. Beginning in March, I studied with Professor Jean-Claude Perriard at the Swiss Federal Institute of Technology, Zurich, Switzerland, and then in October I spent an additional 6 months working with Professors Avri Ben-Ze’ev, Benjamin Geiger, and Alexander Bershadsky at the Weizmann Institute of Science, Israel. Below is a summary of our studies during the past year.

**Forced Expression of Tropomyosin 2 or 3 in v-Ki-Ras-transformed Fibroblasts Results in Distinct Phenotypic Effects**

M. Gimona, D.M. Helfman

Transformation of cells in tissue culture results in a variety of cellular changes including alterations in cell growth, adhesiveness, motility, morphology, and organization of the cytoskeleton. Morphological and cytoskeletal changes are perhaps the most readily apparent features of transformed cells. Although a number of studies have documented a decrease in the expression of specific tropomyosin (TM) isoforms in transformed cells, it remains to be determined if the suppression of TM synthesis is essential in the establishment and maintenance of the transformed phenotype. To address the roles of different TM isoforms in transformed cells, we have examined the effects of expressing specific TM isoforms in transformed cells using a Kirsten-virus-transformed cell line (ATCC NRK 1569) as a model system. In contrast to normal fibroblasts, the NRK 1569 cells contain reduced levels of TM-1 and undetectable levels of TM-2 and TM-3. These cells have a rounded morphology and are devoid of stress fibers. Employing expression plasmids for TM-2 and TM-3, stable cell lines were established from the NRK 1569 cells that express these isoforms individually. We demonstrate that expression of TM-2 or TM-3 leads to increased cell spreading accompanied by the formation of identifiable microfilament bundles, as well as significant restoration of well-defined vinculin-containing focal adhesion plaques, although expression of each isoform exhibited distinct properties. In addition, cells expressing TM-2, but not TM-3, exhibited contact-inhibited cell growth and a requirement for serum. Experiments are in progress to further understand the molecular mechanisms by which expression of specific TM isoforms leads to changes in the cytoskeleton and cell signaling.

**Incorporation of GFP-Tropomyosin Isoforms in Living Neonatal Rat Cardiomyocytes: Nonmuscle TM-4 Requires Cooperative Interactions with Other Low-molecular-weight Isoforms for Thin-filament Binding**

D.M. Helfman [in collaboration with Jean Claude Perriard, Institute for Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland]

The ability of striated muscle (skeletal muscle α-TM) and nonmuscle (fibroblast TMs 1, 2, 3, 4, 5, 5a, or 5b) isoforms of tropomyosin to incorporate into actin filaments of neonatal rat cardiocytes (NRCs) and fibroblasts was studied using GFP, VSV, and HA epitope-tagged expression plasmids. All epitopes were fused to the amino-terminal end of the TM cDNA and analyzed following transient transfections. The expressed fusion proteins were analyzed for l-band incorporation in NRCS and incorporation into microfilaments in fibroblasts. All isoforms, except fibroblast TM-4, were able to incorporate into the l-band of NRCs. In addition, cells that incorporated the muscle or nonmuscle GFP-TMs into their sarcomeres continued to beat and exhibited sarcomeric contraction, demonstrating that incorporation of the GFP-TM did not interfere with myofibrilar function. In contrast, all isoforms of TM analyzed, including TM 4, were able to associate with microfilaments in
fibroblasts. When TM-4 was cotransfected with other low-molecular-weight isoforms of TM (TM-5, TM-5a, and TM-5b), it was able to incorporate into sarcomeres of NRCs. This result was not obtained when TM-4 was cotransfected with high-molecular-weight TMs (TM-2 or skeletal muscle α-TM). These data demonstrate that the ability of TM-4 to bind to actin filaments can be influenced by its interactions with other low-molecular-weight TM isoforms. Furthermore, these studies demonstrate that GFP-TM can be used to study thin-filament dynamics in muscle cells and actin filament dynamics in nonmuscle cells.

Cytoskeletal and Adhesion Proteins in Transformed Cells

D.M. Helfman [in collaboration with Avri Ben-Ze'ev, Benjamin Geiger, and Alexander Bershadsky, Weizmann Institute of Science, Rehovot, Israel]

Malignant transformation is accompanied by a number of alterations in the cytoskeleton and cell adhesion. Using an SV40-transformed human fibroblast cell line, we investigated the distribution of adherens junction molecules as well as the effects of expressing different isoforms of tropomyosin, caldesmon, and small GTPases (rho, rac, and cdc42) on cell shape, microfilaments, focal adhesions, tyrosine phosphorylation, and adherens junctions. Different isoforms of TM were found to exhibit distinct phenotypic effects on the actin cytoskeleton. In addition, low-level expression of caldesmon led to enhanced formation of microfilament bundles, whereas high-level expression led to disruption of microfilament bundles and inhibition of ATP-driven contractility. Expression of active rho, rac, and cdc42 led to the formation of stress fibers, lamellipodia, and filopodia, respectively, demonstrating that the cellular targets for these signaling molecules were functional in these transformed cells.

Distribution of TM isoforms in Dynamic Actin-containing Cytoskeletal Structures

C. Berthier

Several lines of biochemical evidence have suggested that tropomyosin could be involved in the stabilization of actin filaments. The multiplicity of TM isoforms in one single cell (e.g., seven isoforms per fibroblast) raises the possibility that specific associations of given isoforms are required for the distinct actin-based structures. This project was designed with regard to the following questions: (1) Do different TM isoforms achieve their function by binding to spatially distinct subsets of actin filaments, and (2) how is the localization of the TMs restructured during dynamic cellular events. We have constructed chimeric genes encoding the different TMs fused at their amino terminus to green fluorescent protein (GFP). We have been able to establish stable cell lines of 3T3 fibroblasts expressing these different fusion proteins, and using GFP as a reporter, we are currently investigating the subcellular distribution of the GFP-TMs. Preliminary results suggest some differences exist between the localization of different GFP-TM fusions: High-molecular-weight TMs appear, for example, to be better incorporated into actin stress fibers than low-molecular-weight TMs. What is of particular interest is that this cellular system now offers us the possibility to follow the dynamic behavior of the GFP-tagged TMs, using fluorescence time-lapse video recording of living cells. Experiments are now in progress to characterize the dynamic localization of the different TM isoforms during various cellular motility events involving reorganization of the actin cytoskeleton, such as protrusion of filopodia, lamellipodia ruffling, forward movement, spreading and assembly of stress fibers, and cytokinesis.

Using transfection of tagged proteins in cultured cells followed by immunofluorescence, we are also currently investigating the preferential localization of the TM isoforms and the role of caldesmon in normal and transformed cells. Preliminary results strongly suggest that caldesmon helps the binding of the TMs to actin filament bundles. We are in the process of determining if caldesmon has a role in promoting the binding of specific isoforms to actin filaments, as in vitro results suggested. Several reports have shown that expression of TMs is altered in transformed cell lines, and we anticipate that our study will give new insights into the role of TMs in the actin cytoskeleton of cancerous cells.

Identification of Novel Tropomyosin Binding Proteins

A.J. Rai

Tropomyosins (TMs) comprise a family of highly conserved filamentous actin-binding proteins that
have been identified in virtually all eukaryotic cells. To date, there are four TM genes that have been characterized in rat, and these give rise to at least 16 different isoforms. Expression of these isoforms occurs in a tissue-specific manner. The function of TM is best understood in striated muscle, where in conjunction with the troponin complex, TM regulates the calcium-sensitive interactions that modulate actin myosin-based contraction. Smooth muscle cells and nonmuscle cells are devoid of a troponin complex, and these cells express a multiplicity of different TM isoforms, whose functions are poorly understood. The system that we use for our investigations is that of the rat fibroblast. These cells express at least seven different isoforms of TM.

A plethora of genetic and biochemical data suggest that different TM isoforms have different roles in the cell. To delineate these specific functions in vivo, we have sought to identify isoform-specific TM-binding proteins. We have used several different approaches to identify candidates that may serve these roles, including affinity chromatography, coimmunoprecipitation, gel overlay, and the two-hybrid system. One such protein that we have identified recently appears to interact selectively with specific TM isoforms. Current work suggests that this binding is dependent on alternatively spliced regions of the TM molecule. Future experiments are planned to further characterize this binding activity and to determine its physiological relevance in the regulation of cytoskeletal dynamics. This work is not only of interest in basic science, but also of clinical value, as it has long been known that TM gene expression is selectively altered in transformed cells. The identification and characterization of isoform-specific TM-binding proteins may help us to understand how transformed cells maintain their distinct state with reference to cytoskeletal disorganization and other aspects of the transformed phenotype.

Exogenous Expression of Brain Tropomyosin and Tropomodulin Isoforms in Nonneuronal Cells Reveals Their Actin-binding Properties

A. Watakabe, D.M. Hellman

Whereas the roles of tropomyosin and tropomodulin in skeletal muscle are well established, their roles in nonmuscle cells are relatively unknown. We have investigated the properties of neuronal isoforms of tropomyosin and tropomodulin in biochemical and transfection experiments. TMBr1 and 3 are developmentally regulated tropomyosin isoforms expressed in neuronal cells. To examine their binding affinity for F-actin, we produced recombinant TMBrs in bacteria and insect cells and carried out actin cosedimentation assays. We found that TMBr1 and 3 poorly bind to F-actin, unlike other isoforms. To confirm this finding in cultured cells, we transfected TMBr1 and 3 into a rat fibroblast cell line NRK 1570 and examined whether they incorporate into actin-based structures. Consistent with biochemical experiments, the transfected TMBrs stayed mainly in the cytoplasm, although we observed incorporation of TMBrs into some actin-based structures. Surprisingly, when we used cardiac cells for transfection, TMBr1 incorporated efficiently into the sarcomeric structure, whereas TMBr3 did not. This result suggests that binding of tropomyosin to F-actin is controlled not only by its intrinsic affinity to F-actin, but also by the cellular environment. We also transfected two different isoforms of tropomodulin into cardiac cells. It was previously shown that E-tropomodulin is localized at the pointed end of F-actin in sarcomeric structure. We found that N-tropomodulin, which is predominantly expressed in neurons, also locates at the same position in sarcomere. This finding strongly suggests that N-tropomodulin also has a pointed-end capping activity as does E-tropomodulin.

Role of Exon Sequences in Splice Site Selection

M. Selvakumar

Previous studies showed that the 3′ splice site of exon 6 (nonmuscle/smooth muscle exon) of β-TM can be utilized only if (1) a previous splicing event joins exons 6 and 8 together or (2) we mutate either the polypyrimidine tract at the 3′ splice site of exon 6 to a poly(U) stretch or the 5′ splice site of exon 6 to a consensus sequence (Cs). Thus, we showed that both the intron and exon sequences contribute to splice site selection. Studies in other systems such as IgG and troponin T have identified purine-rich motifs as important exonic sequence enhancers (ESE). These ESEs are believed to act as binding sites for splicing
factors such as SR proteins. Examination of sequences within exons 6 and 8 revealed the presence of two ESEs in exon 6 (6-1, 6-2) and three ESEs in exon 8 (8-1, 8-2, 8-3). Substitution mutations of these motifs have shown that they are required for the 3' splice site usage of exon 6. We also see that individual motifs have different effects, with 6-1, 6-2, and 8-2 being the most robust ESEs. We used wild-type and mutant exon sequences as probes for UV cross-linking in an effort to look for RNA:protein interactions. We see differences in binding efficiency for several nuclear factors and are currently in the process of identifying some of these proteins.

When pre-mRNAs containing multiple exons and introns (exons 5 through 9) are used, the major splice product detected in vitro unexpectedly skips exon 6. We divided this long substrate into two with the first half containing exons 5-6 and the second half containing exons 6-7-8-9, with the focus being exon and intron sequences in and around exon 6. We observed that the ESEs in exon 6 substantially improve both 3' and 5' splice site usage when one of the splice sites is weak. However, when both the splice sites are strong (poly[U] and Cs), the ESEs are not required. Thus, ESEs may have a role in bridging factors across the exon and thereby stabilize interactions at weak splice sites such as those commonly found in alternatively spliced exons. We have also shown that ESEs are required for exon 6 inclusion in vivo.

The Mechanism of Blocking the Utilization of a Skeletal Muscle Exon in Nonmuscle Cells

C. Chen

By using the rat β-TM gene, we are studying the mechanisms involved in the regulation of alternative splicing. Our previous data demonstrated that two elements in both intron 6 and exon 7 were responsible for the blocking of skeletal muscle exon 7 in fibroblast. The intron regulatory element (IRE), which is located downstream from intron 6, binds polypyrimidine tract binding (PTB) protein and displaces the binding of a constitutive splicing factor U2 snRNP auxiliary factor to the polypyrimidine tract (PPT), which results in the inability of U2 snRNP to bind to the branch point sequence (BPS). The other element identified is a sequence in skeletal-muscle-specific exon 7. The mutation of this sequence (named ex-1) resulted in the activation of exon 7. This sequence is referred to as GRE, which stands for guanine-rich element. The function of GRE is unknown.

To simplify the system, to concentrate on the
GRE, and to increase the sensitivity of the functional assay, a number of simple substrates have been generated and tested in vitro. A pair of simple substrates designated p5-7 wild type and ex-1 mutant, which consists of exon 5, intron 5, and exon 7, fulfilled the requirement and have been chosen for further studying the nature of GRE. At least three possibilities are responsible for the activation of ex-1 mutation. One possibility is that the mutation creates a strong exonic enhancer. This is unlikely because two random sequence mutations of GRE activate exon 7 as well. Another possibility is the ex-1 mutation disrupts a putative secondary structure as demonstrated in the chicken β-TM. This is also not the case because it did not activate exon 7 when the complementary sequence, which was proposed to complement to the GRE, was mutated; additionally, the splicing of exon 7 was not suppressed when the proposed secondary structure was reformed by mutating both strands to complementary sequences. The third possibility is the binding of a suppressor disrupts the binding of another constitutive splicing factor. To test this hypothesis, UV cross-linking was used and a distinct protein band, migrating at approximately 50 kD, was detected specifically to the wild-type RNA covering the GRE. This activity was enriched, excised from the protein gel, and subjected to microsequencing. We are now in the process of further characterizing this factor.

Analysis of a Multiprotein Complex That Assembles on Cis-acting Sequences Involved in Alternative Splicing of β-TM Pre-mRNA

J. Grossman, M. Meyer

We are using the rat β-TM gene as a model system to study the mechanism of alternative splicing. Previous studies have found that at least one protein, the poly-pyrimidine-tract-binding protein (PTB), specifically interacts with sequences upstream of exon 7 which were shown to be involved in the alternative splicing of β-TM pre-mRNA. To further study the role of PTB in the regulated intron, monoclonal antibodies to PTB were prepared. Anti-PTB antibodies did not inhibit the binding of PTB to RNA. To identify additional proteins that interact with sequences within the pre-mRNA, two different assays were used: (1) 35S-Met-labeled nuclear extracts from HeLa cells were mixed with nonbiotinylated RNAs and the RNA-protein complexes were recovered by immunoprecipitation using monoclonal antibodies to PTB, and (2) 35S-Met-labeled HeLa nuclear extracts were mixed with biotin-RNA containing intron 6 and the RNA-protein complexes were recovered using streptavidin-agarose beads. Identical patterns of proteins were observed using both assays. When RNAs containing intron 6 were coprecipitated with antibodies to PTB, a novel set of proteins were found to coprecipitate. In contrast, addition of RNAs containing introns 5 or 7 gave the same results as precipitations without RNA, indicating that these RNAs are unable to form stable complexes with PTB. These results are in agreement with our previous studies demonstrating that PTB interacts with sequences within intron 6, but not with sequences in introns 5 and 7. Finally, analysis of the proteins that assembled on introns 5, 6, or 7 using biotin-RNA revealed a unique set of proteins that interact with each of these sequences, indicating that different heterogeneous nuclear ribonucleoproteins (hnRNPs) are bound to different regions of the pre-mRNA. Comparison of the proteins that assemble on introns from the α-TM and β-TM genes that utilize distant branch points revealed a common set of proteins that assemble on these introns. These studies identify a set of proteins, in addition to PTB, that are most likely to be involved for distant branch point usage.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector  S. Huang  J. Silverman
P. Lorenz  T. Howard
T. Misteli  J. McCann
P. Mintz

Studies in our laboratory are focused on the structural-functional organization of the mammalian cell nucleus. Our research program evolves around understanding the nuclear organization of factors associated with pre-mRNA splicing and the RNA substrates with which these factors interact. The microscopy core facility has been used extensively over the past year and numerous collaborations are under way with the excellent technical expertise of Tamara Howard.

The Dynamics of Pre-mRNA Splicing Factors in Living Cells

T. Misteli, D.L. Spector

Pre-mRNA splicing factors are distributed nonhomogeneously in the nucleus of mammalian cells. By antibody labeling, they are concentrated in 20–40 distinct nuclear domains, termed speckles. The function of nuclear speckles is unknown. To investigate the behavior of nuclear speckles in vivo, we have visualized splicing factors in the nuclei of living cells. Essential splicing factors were fused to the green fluorescent protein (GFP). When expressed in mammalian cells, the fusion proteins could easily be detected by fluorescence microscopy in living cells. The fusion proteins behaved identically to endogenous pre-mRNA splicing factors and were functional in vivo. Using time-lapse fluorescence microscopy, the dynamic properties of splicing factors were analyzed. Nuclear speckles did not significantly alter their position within the nucleus over long observation periods; however, the periphery of speckles appeared highly dynamic as extensions formed from the edge of speckles, and particles containing splicing factors were frequently seen to associate with and dissociate from speckles. These movements were strictly dependent on ongoing RNA polymerase II transcription and involved dephosphorylation/phosphorylation events as they were sensitive to inhibitors of protein kinases and protein phosphatases. To test whether these extensions occurred directly in response to transcriptional activation of particular genes, cell lines were used that carry stably integrated inducible viral genes. Upon transcriptional activation, pre-mRNA splicing factors were released from speckles and accumulated at the sites of active transcription. These observations suggest that pre-mRNA splicing factors are highly dynamic within the interphase cell nucleus and that they directly respond to transcriptional activation of genes by relocation from speckles to active genes. Further studies will address to what degree the position of speckles within the nucleus is predetermined and what determines the position of speckles within the nucleus.

The molecular mechanisms for these redistributions are unknown, but they might involve protein phosphorylation and dephosphorylation events. To address the role of protein phosphatases, we have developed a permeabilized cell system in which morphological redistributions of pre-mRNA splicing factors observed in vivo can be reproduced in vitro. We have identified a serine/threonine phosphatase-I-like activity as a factor in the modulation of the distribution of pre-mRNA splicing factors. From these observations, we propose a model in which a cycle of phosphorylation and dephosphorylation determines the localization of pre-mRNA splicing factors. We are currently testing this model using biochemical means to analyze the various phospho-isoforms of cellular pre-mRNA splicing factors.
FIGURE 1 Fluorescence and electron microscopic examination of the perinucleolar compartment. The PNC was observed by immunofluorescence in HeLa cells using an antibody specifically recognizing PTB (arrowheads in A). At the electron microscopic level, the PNC (arrowhead) can be observed to be composed of electron-dense, thick short strands measuring 80–180 nm in diameter (B). These strands are surrounded by less electron-dense areas. Some of the strands are in direct contact with the surface of the nucleolus (arrows in B). Bars: (A) 10 μm; (B) 1 μm.

Distribution of Pre-mRNA Splicing Factors and Chromosomal Domains

J. Silverman (Undergraduate Research Program), T. Misteli, D.L. Spector

The distribution of pre-mRNA splicing factors within the nucleus is dependent on the overall level of transcriptional activity of the cell. We set out to test whether local differences in transcription levels within the genome also had an effect on splicing factor distribution. Normal female cells contain two X chromosomes, one of which is transcriptionally active and the other transcriptionally inactive. The two X chromosomes were visualized by chromosome painting techniques, and at the same time, the essential splicing factor SC35 was detected by antibody labeling. Using high-resolution confocal laser scanning microscopy and statistical analysis, we have been able to demonstrate that in the majority of cells, the splicing factor SC35 was associated with only one of the two X chromosomes and that the frequency of association of SC35 with the inactive X chromosome was about twofold lower than its frequency of association with the transcriptionally active X chromosome. These observations indicate that the distribution of pre-mRNA splicing factors is not only dependent on the overall transcriptional level of a cell, but also influenced by local differences in transcription between particular regions of the genome. Extending these findings, we have initiated studies on the relative positioning of various chromosomes with respect to each other in the interphase nucleus.

Dynamic Organization of the Perinucleolar Compartment

S. Huang, D.L. Spector

The perinucleolar compartment (PNC) is a unique nuclear structure, preferentially localized at the periphery of the nucleolus (Fig. 1). Several small RNAs transcribed by RNA polymerase III (i.e., MRP RNA and RNase P H1 RNA) and the polypyrimidine-tract-binding protein (PTB, hnRNP I) have thus far been identified in the PNC (Ghetti et al., *Nucleic Acids Res.* 20: 3671 [1992]; Matera et al., *J. Cell Biol.* 129: 1181 [1995]; Lee et al., *Proc. Natl. Acad. Sci.* 93: 11471 [1996]). To further characterize the PNC, we
have extensively analyzed this structure in fixed cells using light and electron microscopy, as well as in living cells through time-lapse observations.

We have found, through the examination of a large number of human cancer and normal cells, that PNCs are much more prevalent in cancer cells than in normal cells. The PNC prevalence varies among different cancer cell lines, suggesting that PNC prevalence may be correlated with the degree of malignancy. When interphase HeLa cells were fixed and examined using a monoclonal antibody specifically recognizing PTB, we found that the number of PNCs per nucleus appears to be consistent from parental cells to daughter cells, suggesting that PNCs are heritable through cell divisions. When cells were preextracted using a nuclear matrix preparation protocol, the PNC was found to be associated with the insoluble fraction of the nucleus. The PNC dissociates in early prophase and reforms at late telophase in the daughter nuclei prior to the reentry into the nuclei of all the PTB proteins. The reformation of the PNC in daughter cells does not require new protein synthesis, suggesting that the newly formed PNC is composed of proteins synthesized in the previous cell cycle. The earliest detectable PNCs at late telophase appear to be associated with nucleolar organization regions (precursors of the nucleolus). Immunoelectron microscopic examination using a monoclonal antibody specifically recognizing PTB revealed that the PNC is in direct contact with the surface of the nucleolus and that the immunolabeling of PTB appears to be heterogeneous within the structure. A fusion protein between PTB and green fluorescent protein (GFP) was generated to visualize the PNC in living cells. Time-lapse studies revealed that the size and shape of the PNC are dynamic over time. To analyze the sequence requirement for PTB to be localized in the PNC, deletion mutants of the GFP-PTB fusion protein were generated. Results of these experiments showed that at least three RRMs are required for the fusion protein to be targeted to the PNC. As previous studies demonstrated that three RRMs from either the carboxyl or amino terminus of the protein are necessary and sufficient for PTB to bind the pyrimidine-rich internal ribosomal entry site (IRE) in virus-infected cells (Kaminski et al., RNA 1: 924 [1995]), our results would suggest that the RNA-binding capacity of the PTB protein may be necessary for the protein to be localized in the PNC. Studies are under way to analyze other components of the PNC and its function.

### Intracellular Interactions and Dynamics of Antisense Oligonucleotides


Antisense oligonucleotides designed to hybridize to complementary regions in their target RNA bear a great promise for use as specific molecular drugs. A large variety of chemical modifications of the basic nucleic acid structure have been introduced into oligonucleotides to improve their efficacy, especially to enhance their nuclease stability and binding affinity. Whereas the physicochemical properties of the different types of oligonucleotides have been well characterized, their cell biological behavior is far less understood. Our studies are designed to gain insight into the intracellular distribution of antisense oligonucleotides in relation to their antisense activity. We have set out to characterize the interactions of specific oligonucleotides with their target molecules as well as with other cellular components. To do so, we employ 18-20-mer oligonucleotides fluorescently tagged at either terminus, and single cells are evaluated for antisense activity by fluorescence microscopy. Our basic administration method of the oligonucleotides is microinjection into the cytoplasm or nucleus of cells. Injected cells are side by side with uninjected control cells, and microinjection permits us to evaluate the potential of new antisense chemistries which are difficult to introduce into cells otherwise.

We are currently using a model system in which overexpressed rat tropomyosin (TM-1) is our target RNA. The target gene and a control gene, the related rat tropomyosins TM-4 or TM-5, are coinjected with the oligonucleotide to be tested into HeLa cells. Inhibition of expression of the target gene TM-1 while still maintaining the ability to detect expression of the control gene TM-4 or TM-5, by immunocytochemistry, denotes specific antisense activity in a particular cell. Thus far, we have found two sequences in the coding region of TM-1 that led to specific inhibition of TM-1 expression. One sequence is located in exon 5 and the other in exon 9. The fact that the phosphorothioate modification but not the 2' propoxy phosphodiester modification of the same oligonucleotide sequence exerted antisense activity suggested that the antisense effect is RNase-H-dependent.

During our studies of the intracellular dynamics of oligonucleotides, we have established a new property
of phosphorothioate-type oligonucleotides: Oligonucleotides microinjected into nuclei underwent nucleo-cytoplasmic shuttling. We have observed this phenomenon after microinjection of the oligonucleotides into one nucleus of naturally occurring binucleated Ref-52 cells as well as in heterokaryon assays. Shuttling of phosphorothioate oligonucleotides could be abolished by wheat germ agglutinin and by lowering the temperature to 4°C. Therefore, it is a nuclear-pore-mediated process that occurs by facilitated or active transport. We are currently in the process of characterizing in detail the involvement of ATP and GTP, as well as known import/export systems for proteins and RNAs.

Purification and Characterization of Interchromatin Granule Clusters

P. Mintz, D.L. Spector

In the past year, we have been focusing on the purification of interchromatin granule clusters (IGCs) that are enriched in pre-mRNA splicing factors. These structures have been extensively characterized at the immunocytochemical level. IGCs measure between 0.5 and 1 μm in diameter and are composed of 20–25-nm granules that are interconnected by thin fibrils. It has been proposed that the IGCs may serve as storage and/or reassembly sites for splicing factors. Little information is known about the biochemical composition of the IGCs. Therefore, we have used two different starting materials in trying to isolate the IGCs: HeLa tissue culture cells and mouse liver cells. During the isolation procedure, the purity of the IGCs and nuclear residue contamination were monitored by light and electron microscopy.

We have been partially successful in purifying the IGCs from both HeLa tissue culture cells and mouse liver cells. The primary contamination, DNA, was observed in the final stage of the purification procedure. This is promising considering that more problematic nuclear contamination such as the nuclear lamina and internal nuclear matrix were not present in the final fraction. To show that we have purified the IGCs, immunogold labeling was performed with the anti-3C5 antibody (recognizes the family of SR proteins) on the purified IGC fractions and examined using electron microscopy. We have observed structures (IGCs) labeled with the 3C5 antibody in concentrated areas. However, the labeling was not homogeneous throughout the section. In many cases, in a single section, there were one or two areas labeled with the antibody and the majority of the structures were not labeled. We have also performed silver staining and Western blot analysis on all of the fractions obtained throughout the purification steps. We observed numerous bands in the various fractions. The biochemical analysis is still ongoing, and a more extensive biochemical analysis will be performed once we are confident that we have isolated pure IGCs. Our research is promising, and within the next year, we hope to be able to purify the IGCs completely.

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Our laboratory uses the small nematode *Caenorhabditis elegans* as a model organism for the study of basic biological problems. The two main areas that we are currently investigating are (1) programmed cell death (apoptosis) and (2) nervous system function and development. Because important biological processes are evolutionarily conserved, we believe that the general rules that we find in *C. elegans* will also apply to humans.

**PROGRAMMED CELL DEATH**

Programmed cell death (PCD, also known as apoptosis) is a mechanism used by multicellular organisms to eliminate cells that are not needed or are potentially dangerous. PCD has important roles in animal development and homeostasis and occurs in a wide variety of tissues in both vertebrates and invertebrates. Proper control of PCD is crucial: Breakdown in the regulation of this process contributes to the pathogenesis of a large number of diseases, including cancer, autoimmunity, neurodegenerative diseases, ischemic stroke, and myocardial heart infarct.

The nematode *C. elegans* provides an attractive system for the study of PCD, as its development has been extensively characterized and the animal is readily amenable to genetic and molecular manipulations. Hundreds of mutations that affect nematode cell death have been isolated over the years. These mutations identify over 16 genes that function in the regulation and execution of apoptosis; these genes define a genetic pathway for programmed cell death in *C. elegans*. We and other investigators have previously shown that several of these *C. elegans* cell death genes have homologs that perform similar functions in mammalian apoptosis. This conservation in sequence and function of cell death genes between *C. elegans* and mammals strongly suggests that the molecular mechanism for PCD has remained conserved through evolution. Thus, the knowledge gained about PCD in *C. elegans* promises to be relevant to our understanding of apoptosis in human development and disease.

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**Identification and Characterization of CED-9-interacting Proteins**

M. Spector, S. Desnoyers, D. Hoeppner

We have previously found that the cell survival gene *ced-9* is homologous to a family of apoptotic regulators in mammals that include the genes *bcl-2, bcl-x, bax,* and *bak*. The mechanism of action of these genes is not known, but since neither *CED-2, bcl-x,* and *bcl-x* are antagonized by *CED-9*, it is assumed that they act by interacting with other proteins. In *C. elegans*, two good candidate targets are the pro-apoptotic proteins *CED-3* and *CED-4*: Both are essential for programmed cell death and are antagonized by *CED-9*. We therefore tested whether these two proteins could interact with *CED-9*. Interestingly, we found that *CED-9* interacts physically with *CED-4*, both in the yeast two-hybrid system and in vitro. Mutations that reduce or eliminate *CED-9* activity also disrupt its ability to bind *CED-4*, suggesting that this interaction is important for *CED-9* function. Thus, *CED-9* might control *C. elegans* cell death by binding to and regulating *CED-4* activity. Because the pathway for programmed cell death is conserved from *C. elegans* to mammals, we suspect that mammalian Bcl-2 family members might, in a similar fashion, control apoptosis through interaction and regulation of *CED-4* homologs or analogs.

To complement these directed experiments, we have also performed a general screen for *C. elegans* proteins that interact with *CED-9* in the yeast two-hybrid system. We have identified several protein fragments that appear to interact specifically with full-length *CED-9*. We are in the process of further characterizing these clones to determine their possible involvement in apoptosis.
Programmed Cell Death in the C. elegans Germ Line
S. Milstein, T. Gumienny

PCD has a major role in the germ line. Germ cells are by far the most common cell type to undergo PCD: During development, 131 somatic cells die; in contrast, more than 300 germ cells die during adulthood. Thus, during an animal's lifetime, more PCDs occur in the germ line than in all the somatic tissues combined. Most, but not all, mutations that affect PCD in the soma also affect germ cell death. ced-3 and ced-4 are both required for germ cells to die, whereas ced-9 is required to protect oocytes from death: In the absence of ced-9, too many germ cells die, resulting in sterility. In contrast, genes that affect PCD only in specific somatic cells (such as the ces genes and egl-1) do not affect germ cell death.

To understand how germ cells make the decision between life and death, we are screening for mutations that specifically affect PCD in the germ line. In the last year, we have isolated a number of such mutations, which we are now characterizing. We plan to continue with our screens in the coming year, as none of the approaches used so far have been saturated.

Genes Involved in the Engulfment of Dying Cells
Q. Liu, T. Gumienny

Once a cell activates the apoptotic machinery, a number of downstream "subprograms" are activated, with the goal of rapidly breaking down the cell and removing it from the body. These downstream programs are responsible for the morphological and biochemical changes that are characteristic of apoptotic cells. One important subprogram results in the generation of signals that promote recognition and phagocytosis of the dying cell by other cells.

Six genes have previously been shown to function in this process in C. elegans. Genetic analysis has suggested that these six genes identify two partially redundant pathways. In the past year, we have identified several additional phenotypic criteria by which these genes can be distinguished, strongly supporting the original classification into two groups. We have also made great progress in our effort to clone ced-6, one of these engulfment genes. We have successfully rescued ced-6 mutants with genomic DNA from a wild-type animal and have localized the rescuing activity to a single genomic cosmid. We are now in the process of determining which of the genes present on this cosmid corresponds to ced-6. Finally, we have identified a seventh gene involved in the engulfment process (ced-12) and have begun to characterize it at the genetic level.

Understanding the nature of the engulfment-promoting signals and how they control phagocytosis should significantly further our knowledge of the events that surround and follow the activation of the cell "deconstruction" program by the apoptotic cell. Rapid recognition and phagocytosis of apoptotic cells is also an important aspect of programmed cell death in vivo, as unengulfed apoptotic bodies can undergo secondary necrosis, leading to inflammation and possibly encouraging the development of autoimmune disease.

NERVOUS SYSTEM FUNCTION AND DEVELOPMENT
A second area under investigation in the lab is the nervous system in C. elegans. The small size and relative simplicity of the nematode nervous system allows us to address complex questions readily at the genetic and molecular levels.

Development of a Tetracycline-responsive System in C. elegans
S. Tharin, G. Parvilus

A tetracycline-responsive system for conditional gene expression has recently been developed for use in mammalian cells in culture and in transgenic mice. Because such a conditional expression/repression system would be useful for our studies of the nervous system, we have been working toward adapting this system for C. elegans. In our preliminary experiments, we attempted to control gene expression in the six touch cell neurons. Our system consisted of two transgenes. The tetracycline-controlled trans-activator, which encodes a chimeric protein composed of a portion of the tet repressor protein from Escherichia coli fused to the transcriptional activation domain of the viral protein VP16, was placed under the control of the mec-7 gene, which drives expres-
sion in the touch cells. The tetracycline-responsive reporter consisted of lacZ placed under the control of the pes-10 minimal promoter, upstream of which we inserted many copies of the tet operator. We generated a large number of transgenic lines containing various ratios of trans-activator and reporter constructs and tested them for lacZ expression in the presence or absence of the tetracycline analog dauxocycline. Although our initial results with these lines were disappointing (strong background staining, and little if any specific staining), we are still bullish on this approach. We are now experimenting with different coinjection markers and stronger promoters driving the tetracycline-controlled trans-activator and hope to get this system working in the coming year.

Learning and Memory

S. Tharin [In collaboration with J. Connolly and T. Tully, Cold Spring Harbor Laboratory, and D. van der Kooy, University of Toronto, Canada]

We have recently started a research program on the molecular basis of associative learning and memory in C. elegans. We are pursuing this project in collaboration with the Tully and van der Kooy laboratories. During the last year, we have made some progress toward the development of a satisfactory conditioning paradigm, which we will use to screen for mutants defective in acquisition, short-term memory, or long-term memory. Such mutants will then be characterized at the behavioral, genetic, and molecular levels.

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GENETICS

The inescapable news in the world of genetics during the last year was the announcement of the cloning of a sheep in Scotland. Although this work was foreshadowed by many years by that of Briggs and King in Indiana and of Gurdon at Cambridge who had cloned amphibians, the near identical reproduction of a mammal galvanized the world's press and invited the inevitable questions. Whether the cloning of humans will ever happen, except as a consequence of the fairly common natural accident of identical twins, cannot be said. However, no technical advance has ever been left unexploited, that mankind viewed to be in our best interest, whether at the time we were dead right or dead wrong.

EUKARYOTIC CELL CYCLE CONTROL

D. Beach  S. Allan  D. Conklin  J. Hudson  M. Serrano
M. Caligiuri  K. Dai  R. Maestro  P. Sun
A. Carnero  K. Galaktionov  K. Okamoto  J. Wang
J. Chen  J. Hoffman  S. Salghetti  L. Xie

During the course of the year, Maureen Caligiuri accepted a position as Scientist at Mitotix, Inc., in Cambridge, Massachusetts; Johannes Hofmann went to the IMP in Austria; Koji Okamoto joined Carol Prives’ lab at Columbia University; and Jim Hudson accepted a lectureship at the Institute of Child Health in London. We were joined by two new postdoctoral fellows, Dr. Amancio Carnero and Dr. Jenny Wang.

Induction of the p16 Tumor Suppressor in Response to Oncogenic Stimuli

M. Serrano, D. Beach

The p16^{INK4a} gene is a tumor suppressor mutated in a wide variety of cancers. Protein p16 associates with CDK4 and inhibits the G_{1} cyclin-dependent kinase CDK4/cyclin D. We have previously shown that p16-deficient mice are viable but are highly prone to spontaneous and carcinogen-induced tumors, thus directly implying a role for p16 in tumor suppression. Little is known about the regulation of p16 or about circumstances in which p16 might act to promote cell cycle arrest. An important clue to investigate the regulation of p16 came from our observation that primary fibroblasts derived from p16-deficient mice can be tumorigenically transformed by oncogenic ras, whereas transformation of wild-type fibroblasts requires an additional cooperating oncogene.

On the basis of the above observations, we have hypothesized that p16 is induced by some signal or condition specifically present during tumorigenesis, but not during normal cell physiology. We have found, in collaboration with Scott Lowe here at the Laboratory, that expression of oncogenic ras in primary human or rodent cells induces the accumulation of p53 and p16 and results in a permanent G_{1} arrest. The arrest induced by ras is accompanied by, and is phenotypically indistinguishable from, cellular senescence. The causal role of p53 and p16 in mediating this protective response is implied by the fact that cells derived from p16-deficient mice or from p53-deficient mice are permissive to the presence of oncogenic ras, and, indeed, these cells become morphologically transformed upon introduction of ras. We propose that p16 and p53 act to prevent cellular transformation by abnormal mitogenic stimuli such as oncogenic ras. This experimental system will allow us to analyze the regulation of p16 expression...
and to dissect the relevant stimuli that induce p16. An additional implication of these results is that cellular senescence can be prematurely activated and is therefore not simply a reflection of the accumulation of cell divisions.

**cdc25 Cell Cycle Phosphatase as a Target of c-myc**

K. Galaktionov, X. Chen, D. Beach

The c-Myc proto-oncogene (Myc) belongs to a family of related genes implicated in the control of normal cell proliferation and the induction of neoplasia. More recently, Myc has been implicated in the induction of apoptosis or programmed cell death. Myc-induced apoptosis is enhanced by growth factor deprivation, and the oncogenic-apoptotic switch is sensitive to the level of mitogenic stimulation. Myc, in partnership with Max, forms a transcription factor. Myc activity in cell cycle progression and apoptosis requires the presence of both intact trans-activation and Max-binding domains. Few transcriptional targets of Myc have been identified. Recently, it has been shown that induction of Myc in growth-factor-depleted cells causes rapid activation of the cyclin E/cdk2 kinase without alteration in the abundance of cdk2 or cyclin E. In this situation, although cdk2 is inactive, it is phosphorylated at the essential Thr-160 residue prior to Myc induction, suggesting that activation of cdk2 may be mediated either by dephosphorylation of the Thr-14 or Tyr-15 residues or by loss of protein inhibitors such as p21, p27, or p16. These observations prompted us to evaluate whether cell cycle activators such as cdc25 genes might serve as direct transcriptional targets of Myc.

Using cells expressing estradiol-inducible mycER fusion protein, we have shown an increase in the level of cdc25A RNA and protein closely following induction of mycER with b-estradiol. On the contrary, cdc25A was not induced by the nononcogenic mutant (A 105-143) of Myc. To investigate whether the regulation of cdc25 by Myc might be direct, we utilized a modified precipitation assay to search for Myc/Max-binding sites within genomic human cdc25A, B, and C DNA clones. The cdc25A gene contains three Myc/Max-binding sites within the first two introns. One putative Myc-binding site was found in cdc25B. These can direct Myc-dependent transcription from a heterologous promoter. Surprisingly, in the absence of adequate levels of growth factors, introduction of cdc25A into a responsive cell line causes apoptosis. Myc-driven apoptosis was inhibited by cdc25A antisense oligonucleotides, suggesting that cdc25A expression might be essential for apoptosis under these experimental conditions. Finally, inhibition of cdc25A expression using the cdc25A genetic suppressor element (GSE) expressed from a retroviral vector also resulted in the inhibition of Myc-driven apoptosis. These findings suggest that cdc25A is a physiologically relevant transcriptional target of c-Myc.

**Identification of Protein Ubiquitin Ligases Involved in the Proteolytic Degradation of Cdc25 in Mammalian Cells**

M. Caligiuri, D. Beach

The Cdc25 phosphatases are potential human oncogenes whose levels are elevated in a large number of breast cancers. Understanding the biochemical mechanism of their regulation is therefore of fundamental importance. Cdc25 has been shown to be ubiquitinated in both fission yeast and mammalian cells (Nefsky and Beach 1996; T. Kim and D. Beach unpubl.). Polyubiquitination involves three successive reactions in which ubiquitin is activated by the ubiquitin activating enzyme, E1, in an ATP-dependent reaction and then transferred via a thiolester bond to a ubiquitin conjugating enzyme (UBC), E2. The molecule of ubiquitin is then transferred through a thiolester intermediate involving a ubiquitin protein ligase, E3, to a lysine residue on the target protein. The ubiquitinated products are then degraded by the 26S proteosome. The specificity of the reaction is thought to be rendered by the interaction between the E3 enzyme and the target protein.

The fission yeast pub1 gene has been shown to be involved in the ubiquitination of Cdc25 (Nefsky and Beach 1996). Pub1 shares a high degree of amino acid sequence similarity with E6-AP, the E3 involved in the ubiquitination of the p53 tumor suppressor. We have used the sequence of pub1 to search the expressed sequence tag database to identify human genes that have homology with the active site region of the E3 enzymes. In this way, we identified five
new genes encoding human protein ubiquitin ligases, hpub1 through hpub5. The sequences obtained from the database were then used to clone the corresponding cDNAs by hybridization. The proteins encoded by two of these genes, hpub1 and hpub2, share a high degree of amino acid sequence homology with yeast Pub1 and were chosen for further analysis. Whereas the sequence conservation between Pub1 and E6-AP is confined predominantly to the carboxy-terminal third of the protein in the so-called "hect domain," that with hPub1 and hPub2 extends about 100 residues toward the amino terminus. Interestingly, this region has been shown to be involved in the interaction between E6-AP and the E6 protein and may thus define an interaction domain for the Pub proteins.

Using whole-cell extracts prepared from HeLa cells, we have shown that recombinant human Cdc25A can be ubiquitinated in vitro in the presence of an ATP regenerating system. This assay utilizes biotinylated ubiquitin for the visualization of polyubiquitinated products with horseradish-peroxidase-conjugated streptavidin. Titration of the extract allowed the lower limit of the reaction to be defined. We performed the reaction with a limiting amount of extract and found that the addition of recombinant E1, hPub2, and UBC4 restored the polyubiquitination of Cdc25A. We found that although the extract could not be completely omitted from the reaction, it could be titrated to the level at which polyubiquitination of Cdc25 was not detected in the absence of UBC4 and hPub2. These results suggest that hPub2, UBC4, and an as yet unidentified protein(s) are involved in the degradation of Cdc25. We believe that phosphorylation of Cdc25 may precede its degradation and that the extract is supplying the protein kinase responsible for this modification.

PUBLICATIONS


In Press

Historically, many complex biological problems have surrendered first to attacks that are uniquely available in genetically tractable organisms. For example, Drosophila provided the first clues to the role of homeodomain proteins in regulating pattern formation during development, and the yeasts played a critical part in unraveling the basic mechanics of cell cycle control. However, many biological questions are best addressed directly in mammalian cells in which genetic approaches have generally been lacking. Efforts during the past year have focused on the development of technologies that will allow genetic approaches to be used in animal cells.

The genetic system that we have developed is conceptually modeled upon the genetic techniques that are available in yeast. These include (1) the ability to deliver complex gene libraries, (2) the ability to distinguish those phenotypes that are created upon the delivery of a specific gene from phenotypic variants (revertants) that preexist in a cell population, (3) the ability to efficiently recover genes from a phenotypically selected cell, and (4) the ability to reintroduce recovered genes into cells to confirm their ability to elicit a phenotype.

The design of the system had to compensate for the unique difficulties of manipulating animal cells in culture. Two major barriers have hindered past attempts at genetics in animal cells. The first was the inability to manipulate animal cells to create mutants. Unlike yeast, animal cells do not offer the opportunity of working with a haploid form, and unlike Drosophila and Caenorhabditis elegans, crosses cannot be used to create homozygous mutations in convenient genetic backgrounds. Therefore, the phenotypes that are available in animal cells tend to be less "tight" (i.e., to have a higher reversion rate) than those that are available in a well-constructed yeast or Drosophila mutant. The second problem was one of mass. Although it is relatively straightforward to analyze a very large number of yeast cells, the ability to screen animal cells is more limited.

A number of criteria had to be met to overcome these problems. The efficiency of gene transfer into animal cells had to be extremely high in order to allow the screening of high-complexity libraries without the need to maintain impossibly large numbers of cells. This criterion was met by the use of a retroviral gene delivery system which could achieve rates of stable gene transfer that routinely ranged from 10% to 100%. Since retroviral gene transfer results in the integration of a gene (provirus) into the host genome, a mechanism to distinguish revertants from true phenotypic rescues had to allow removal of the provirus from the genome. This was accomplished by the inclusion of recombinase target sites that flank the integrated provirus. Thus, delivery of a recombinase to the genomic DNA in vivo allows excision of the provirus which is lost from a proliferating cell population by dilution. The excised provirus was constructed to contain both a selectable marker and a bacterial origin of replication. In this way, the excised virus could be recovered as a plasmid in bacteria. The design of the vector allows the excised provirus to be used directly for the production of retroviruses (in a retroviral packaging cell line that we have constructed) for subsequent rounds of screening.

Variations of our vectors have been designed both for the delivery of sense expression libraries and for the delivery of antisense fragment libraries. Both of these vector configurations have been validated through model genetic screens in which they performed quite well. The genetic system is now being applied to aspects of cellular growth control (such as the control of cellular mortality) that have proven intractable with conventional approaches.

**PUBLICATIONS**


Our main interest continues to be the regulation of Start and mitosis in *S. cerevisiae*. We are most interested in the tethering of division to cell growth and in the generation of the basic cell cycle oscillation. Many key cell cycle events are regulated by protein kinase complexes formed between Cdc28 and one of nine cyclins. These fall into two broad groups: the G, cyclins (Cln1, Cln2, and Cln3) that regulate Start and the mitotic B-type cyclins (Clb1, Clb2, Clb3, and Clb4). Two other cyclins, Clb5 and Clb6, not only are very important for DNA replication, but also have roles at Start and perhaps also in early mitosis.

A second interest is yeast telomerase. We have found telomerase activity in yeast and found that a protein called Est1 is associated with the activity. Last year, we found a protein called Bdfl that binds to Est1 and affects telomere length and telomeric silencing. We also found many mutants affecting yeast telomeres.

### Critical Thresholds of G1 Cyclins for Start

B. Schneider

We are interested in knowing how much G1 cyclin is required for Start. Is a critical level required? Or will a small amount suffice if given long enough to work? As a first step, we constructed a strain with genotype *cln1 cln2 cln3 gai-CLN1 gal1* in which very small amounts of galactose can be used to turn on the *GAL-CLN1* construct, and the amount of expression is proportional to the amount of galactose used. With this titratable promoter system, we find that at certain low levels of Cln expression, cells do not go through Start even after many hours, whereas at slightly higher levels of expression, Start occurs efficiently. This strongly supports critical threshold models. An additional finding is that the critical threshold is much lower in larger cells than in smaller cells. Thus, the critical size threshold for Start may have two components: First, as cells become larger, they express more Cln, and second, as cells become larger, they require less Cln to pass through Start. The threshold may decrease with increased size partly because the amount of Sic1, a target of Cln-Cdc28 complexes, decreases in abundance as G1 progresses.

We have also investigated the relationship between growth rate and Cln expression. Because Clns are unstable proteins, their steady-state abundance should reflect their rate of synthesis, and thus there might be less Cln at low growth rates. Analysis of cells grow-
ing at different rates shows that there is in fact a very large difference, tenfold or more, in the amount of Cln at different growth rates. For Start, slowly growing cells need much less Cln than do rapidly growing cells. It seems that the threshold requirement for Cln is quite variable under different conditions. We do not yet understand the nature of the threshold, or why it varies.

Substrates of Cdc28

G. Sherlock

We have done a computer screen of the *S. cerevisiae* genome to find proteins with multiple clustered consensus sites for Cdc28 phosphorylation. Many of the proteins found in this screen are known to have a role in the cell cycle. We are mutating the sites in some of these proteins to see if there is a phenotype. So far, we have examined site mutants in Cin8, a kinesin-like motor protein; Chs2, a chitin synthase; and in a number of proteins involved in DNA replication. The DNA replication proteins include Orc2, Orc6, Cdc6, Mcm3, and Cdc54 (which encodes an Mcm protein). Some of the replication protein site mutants dramatically decrease the efficiency of origin usage, and some combinations of site mutants are lethal. This is consistent with the idea that phosphorylation of these sites, possibly by Cdc28, is important for regulation of DNA synthesis.

In the future, we will see whether the observed phenotypes are indeed due to a lack of phosphorylation or to other defects associated with the amino acid change.

Analysis of WHI3, a New Size Control Gene

T. Volpe

The *whi3* mutation was isolated some years ago, and we are continuing its characterization. It contains an RNA-binding motif, but otherwise has little similarity to other proteins. Like other *whi* mutants, it gives a small-cell phenotype. This seems to be because *whi3* mutants overexpress *CIN1* and *CIN2* G1 cyclins. Overexpression of WHI3 turns off *CIN1* and *CIN2* and is lethal. However, the level at which Whi3 interferes with *CIN* expression is still unclear. Although *GAL-WHI3* does turn off *CIN1* and *CIN2* expression from their own promoters, it does not interfere with *CIN1* or *CIN2* expression from a *GAL* promoter. Even so, neither *GAL-CIN1* nor *GAL-CIN2* rescues the lethality of *GAL-WHI3*. We examined the kinase activity associated with *CLN2* in a *GAL-WHI3* strain and found that it had an unusual array of substrate proteins associated with it. A change in the Cln-Cdc28 kinase complex may be the reason for the effects of *GAL-WHI3*. Whi3 may be used by the cell to direct Cln-Cdc28 kinase to different targets. Some of these may be involved in pseudohyphal growth, since G. Fink and co-workers (pers. comm.) have found that *whi3* mutants are defective in pseudohyphal growth.

Mechanisms of Transcriptional Activation by Cyclin-Cdc28 Complexes

H. Wijnen

One effect of the Cln3-Cdc28 complex—perhaps the only effect—is to induce transcription of a large family of genes involved in the G1/S transition. These include *CIN1, CIN2, CLB5, CLB6, RNR1*, and many genes involved in DNA synthesis and cell wall synthesis. We are trying to discover the mechanism of induction. The promoters of the *CIN3*-inducible genes all include binding sites for the Swi4 transcription factor or for its close relative Mbp1. The Swi4 and Mbp1 DNA-binding proteins each form a complex with another protein called Swi6; these two complexes are called the SBF or MBF transcription factors, respectively. We have found that *swi6* mutants are completely defective for *CIN3*-induced transcription, and this argues that Swi6 is the direct or indirect target for Cln3.

There are three ideas for how Cln3 might activate Swi6 for transcription. First, Cln3-Cdc28 might phosphorylate Swi6, which has several Cdc28 consensus sites. This does not seem to be the case, because mutants of Swi6 lacking all Cdc28 sites have no phenotype. Second, Cln3-Cdc28 might bind to Swi6 and then, while bound, activate transcription by
phosphorylating other molecules. So far, we have not been able to detect Cln3-Swi6 interaction in immunoprecipitation experiments. Finally, Cln3-Cdc28 might phosphorylate and activate an activator of Swi6 or it might phosphorylate and inhibit an inhibitor.

We have looked for high-copy suppressors of mutants expressing very low levels of CLN3 to screen for activators of Swi6. This screen has yielded Phdl, a Swi6-like transcription factor, and Rme1, which can activate transcription of CLN2, and a number of other genes, some of which may function as co-activators of Swi6 with Cln3. Other genetic screens are in progress to identify inhibitors of Swi6.

Telomerase
K. Hidaka, B. Steiner, M. Lessard

Last year, we showed that the Est1 (Ever shorter telomeres) protein was associated with telomerase activity and with the yeast telomerase RNA.

To find other components of telomerase, we did a two-hybrid screen with Est1 that yielded 17 different genes, most of which seem uninteresting. However, one of the interactors, Bdf1, has telomere-related phenotypes. Bdf1 is a bromodomain protein and so may be a component of chromatin. The bdf1 mutant has short telomeres and is defective in telomeric silencing, but not defective in silencing at HML or HMR. There is preliminary evidence of Bdf1-Est1 interaction in immunoprecipitation experiments. Although Bdf1 is not likely to be a catalytic component of telomerase, it could help direct telomerase to the chromosome ends or help regulate it in some way.

We have also screened for new mutants defective in telomere addition and telomere maintenance. Several hundred candidate mutants were obtained, and several dozen of these show senescence. At least some of these also show the continuous telomere shortening phenotype of estl. These are now being placed into complementation groups.

Finally, we have started a three-hybrid screen for proteins interacting with the telomerase RNA. Improvements have been made to the three-hybrid screen to improve specificity, and a number of interesting proteins have been found that interact with telomerase RNA, but not with control RNAs. These are being tested as telomerase components.

Cell Cycle Exit and Stress Resistance
B. Elliott

Previously, we identified TPS2, which encodes trehalose-6-phosphate phosphatase, as a gene needed for stress resistance in yeast. Yeast are known to accumulate trehalose in response to stress, but it has been controversial whether or not the trehalose is a cause or an effect of the stress-resistant state. Our tps2 mutant is stress-sensitive and accumulates much less trehalose than a wild-type cell, but it also accumulates trehalose-6-phosphate (tre-6-P). Sugar phosphates are often toxic, and tre-6-P is known to be an inhibitor of glycolysis. Therefore, we considered the possibility that the stress sensitivity of the tps2 mutant was due to accumulation of tre-6-P. We compared tps1 mutants (which are defective in the first step of trehalose synthesis and do not make tre-6-P) with tps1 mutants and tps1 tps2 double mutants. All three were stress-sensitive, but the tps2 single mutant was significantly more sensitive than the other two. Furthermore, a novel gene isolated as a partial suppressor of tps2 encodes a phosphomutase, and partially converts tre-6-P into trehalose. These results suggest that while part of the stress sensitivity of tps2 is due to the accumulation of tre-6-P, another part is genuinely due to the lack of trehalose. Thus, we believe trehalose is a stress-protectant.

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In Press
Our research mostly focuses on how growth signals cause cells to grow and enter the cell division cycle. For most eukaryotic cells, it is during $G_1$ that decisions are made as to whether or not to divide. For our studies, we use the model organism *Saccharomyces cerevisiae*, which is commonly called budding yeast. Budding yeast cells that have executed the late $G_1$ event(s) termed Start are committed to initiate DNA synthesis, form a bud, and divide. Start is the point where growth signals and mating pheromones control entry into the cell division cycle. The execution of Start seems to require some threshold level of $G_1$ cyclin/CDC28 kinase activity, which is determined in large part by the rate and levels at which the $G_1$ cyclin RNAs accumulate during late $G_1$. However, very little is known about the mechanisms that determine if $G_1$ cyclin RNAs accumulate and the rate at which $G_1$ cyclin RNAs accumulate.

Many cell cycle events are controlled by the regulation of the phosphorylation of certain key proteins. Protein phosphatases are proteins that remove phosphate groups from phosphoserine, phosphothreonine, or phosphotyrosine residues in other proteins. In many cases, removal of the phosphate activates the protein substrate. In other cases, removal of the phosphate inactivates the protein substrate. Most of our effort is focused on the SIT4 phosphatase. We have found that SIT4, which is a type-2A-related phosphatase, is required during $G_1$ for the execution of Start, for bud formation, for the initiation of DNA synthesis, and for spindle pole body duplication. SIT4 is required for the execution of Start because it is required for the expression of the $CLN1$ and $CLN2$ $G_1$ cyclin genes. $CLN1$ and $CLN2$ proteins bind to CDC28, thereby activating the kinase activity of CDC28. When some threshold level of $CLN/CDC28$ kinase activity is achieved, Start is executed and the cells are committed for DNA synthesis and the completion of the cell cycle. Our major goals are to determine how growth signals control the ability of SIT4 to promote Start and bud initiation and to determine the downstream targets of SIT4 that function for Start and bud initiation.

**Function and Regulation of the SIT4 Phosphatase during the Cell Cycle**


SIT4 is a protein phosphatase catalytic subunit whose levels are constant throughout the cell cycle. Therefore, the activity of SIT4 might be regulated in the cell cycle by association with regulatory subunits. In $G_1$ daughter cells, SIT4 exists mostly as monomeric uncomplexed SIT4. During late $G_1$, at a time close to Start (when SIT4 is required for $G_1$ cyclin expression), SIT4 associates in separate complexes with three high-molecular-mass phosphoproteins, termed SAP155, SAP185, and SAP190 (SIT4-associated protein, apparent mass in kilodaltons). Near or just after mitosis, SIT4 is again found primarily as monomeric uncomplexed SIT4.

SAP155 and SAP190 were purified, and R. Kobayashi obtained partial peptide sequences for both proteins. This information was used to clone the *SAP155* and *SAP190* genes. The predicted SAP155 and SAP190 proteins show no significant homology with other proteins in the current databases. However, SAP155 and SAP190 are homologous to each other, with about 30% identical amino acids over the entire lengths of the proteins. Moreover, we also identified two additional *SAP* genes in budding yeast by homology: *SAP185* from the budding yeast genome project and another *SAP* gene by polymerase chain reaction (PCR) using degenerate primers. We have shown that *SAP185* encodes a 185-kD protein that associates with SIT4. We currently do not know if SAP4 associates with SIT4, although we suspect that it does. All four SAPs are acidic.

With the *SAP* genes in hand, we could determine if the SAPs function positively with SIT4 (and turn SIT4 on near Start) or if they function negatively with SIT4 (and turn SIT4 off near Start). Three lines of evidence show that the SAPs function positively with SIT4. First, all four *SAP* genes in high copy
number suppress the temperature-sensitive phenotype of sit4-102 strains. Second, the phenotypic effects due to the loss of all four SAP genes are identical to the loss of SIT4. Third, in terms of the genetic interactions with deletion of CLN3, BEM2, SIS2, or PHO85, the loss of the SAPs is the same as the loss of SIT4. These findings indicate that the SAPs are required for SIT4's cellular function. The loss of the SAPs is not at all similar to the effects due to overexpression of SIT4.  

By sequence similarity, the SAPs fall into two groups: the SAP4/SAP155 group and the SAP185/SAP190 group. The effects due to deletion of various combinations of the SAP genes show that this sequence similarity is also a functional grouping of the SAPs. Further analysis shows that the SAPs (and hence SIT4 also) are providing two functions: one function provided by SAP185 and SAP190 and another function provided by SAP155 (perhaps along with SAP4). Deletion of SAP185 by itself or deletion of SAP190 by itself causes no decrease in the growth rate of the cells. However, deletion of both SAP185 and SAP190 causes a slow growth rate phenotype. Importantly, although either SAP185 or SAP190 on either a low-copy-number cen plasmid or a high-copy-number plasmid restored the growth rate of a Δsap185 Δsap190 strain to wild type, neither SAP4 nor SAP155 could stimulate the growth rate of this strain. Therefore, SAP155, even when overexpressed, cannot provide the function provided by SAP185/SAP190. In addition, the overexpression of SAP185 or SAP190 cannot stimulate the slow growth rate of Δsap155 strains. Therefore, the SAPs are providing at least two cellular functions.

We have prepared antibodies that specifically recognize SAP155, SAP185, or SAP190. With the tools (antibodies and mutations in the genes) to analyze SAP155 and SAP190 in hand, we can now address the following important questions. Do SAP155 and SAP190 regulate, positively or negatively, the activity of SIT4 toward its in vivo substrates? Are SAP155 and SAP190 substrates of SIT4? Interestingly, SAP155, SAP185, and SAP190 become hyperphosphorylated in the absence of SIT4 function. How is the association of SIT4 with SAP155, SAP185, and SAP190 regulated? Via phosphorylation of the SAPs? How do growth signals and cell cycle signals regulate the association of SAP155 and SAP190 with SIT4? We have found that the amount of the SAPs that are associated with SIT4 is regulated not only by cell cycle position, but also by cellular growth signals: by carbon source (glycerol/ethanol versus glucose), by the presence or absence of amino acids in the growth medium, and during saturation of the culture. Possibly, the SAPs are involved in transducing nutrient growth signals via their association with SIT4, thereby linking nutrient growth signals with G1 cyclin expression, bud formation, and other late G1 processes. The elucidation of such a signal transduction pathway will require the determination of how the association of the SAPs with SIT4 is regulated and what downstream processes are regulated by the SAPs and SIT4.

**SIT4 Is Required for G1 Cyclin Accumulation**

K. Arndt, C.J. Di Como

SIT4 is required for the execution of Start because it is required for the expression of the CLN1 and CLN2 G1 cyclin genes. CLN1 and CLN2 proteins bind to CDC28, thereby activating the kinase activity of CDC28. When some threshold level of CLN/CDC28 kinase activity is achieved, Start is executed. During early G1, the levels of CLN1 and CLN2 RNAs are very low. During late G1, the levels of CLN1 and CLN2 RNAs increase at a very rapid rate. The rate at which CLN1 and CLN2 RNAs increase is determined in part by the activity of the CLN3 gene, which encodes a cyclin-like protein. Hyperactive alleles of CLN3 cause a more rapid rate of increase of CLN1 and CLN2 RNA levels, whereas loss-of-function alleles of CLN3 cause a slower rate of increase.

Much evidence shows that CLN3 and SIT4 provide additive pathways for the activation of CLN1 and CLN2 expression. In fact, mutation of both SIT4 and CLN3 causes an essentially lethal effect. Moreover, this lethal effect is due only to a defect in G1 cyclin expression because it is completely cured by expression of CLN2 from a SIT4-independent promoter. Like a sit4 mutation, a mutation in any gene required in the SIT4 pathway for G1 cyclin expression should also be lethal in combination with a cln3 mutation. We used the colony sectoring assay to isolate 400 mutants that could not grow in the absence of CLN3. These mutants, termed ctr for CLN three requiring, fell into 12 complementation groups. Previously, we had analyzed CTR7 in the most depth. The CTR7 gene is the same gene as BCK2, which was originally isolated as a gene that in high copy number can suppress the temperature-sensitive cell lysis defect of
mutations in the protein kinase C pathway. Our analysis had shown that BCK2 and CLN3 function in pathways parallel to CLN1 and CLN2 G₁ cyclin expression.

During the last year, we have focused on CTR9. Cells lacking CTR9 are viable, but they grow slowly at 30°C and are not able to grow at 37°C. Moreover, at 30°C, the ctr9 mutants have a very strong defect in the ability to accumulate CLN1 and CLN2 G₁ cyclin RNA in G₁. Importantly, the slow growth rate of ctr9 mutants is dramatically stimulated to near wild-type rates by expression of CLN2 protein from the ADH promoter or the CLN1 protein from the GAL1 promoter. Therefore, a primary defect in ctr9 mutants is the inability to accumulate G₁ cyclins. Interestingly, CTR9 has 33-amino-acid TPR repeats and is highly similar to a mouse protein that interacts with SH2 domains. We suggest that CTR9 functions in a signal transduction pathway leading to G₁.

TAP42: An Essential 42-kD Protein That Interacts with Both SIT4 and the Type-2A Catalytic Subunits
C.J. Di Como, K.T. Arndt

To identify cellular substrates and/or regulatory subunits of SIT4, we isolated genes that, when present on a high-copy-number plasmid, suppress the temperature-sensitive phenotype of a sit4-102 mutant. From this scheme, we isolated three genes. One gene is SAP155, which encodes the 155-kD subunit of SIT4 (see above). High-copy-number SAP190 also suppresses the sit4 mutant, but we did not isolate SAP190 from this screen because SAP190 is not present in the high-copy-number library. The second gene is HCS26. HCS26 encodes a G₁ cyclin that binds not to CDC28, but to the CDC28-related kinase PHO85. Other cyclins (CLN1, CLN2, ORFD, and CLB5) in high copy number are not able to rescue the temperature-sensitive phenotype of the sit4 mutant. In addition, suppression of the sit4-102 strain by over-expression of HCS26 requires PHO85, suggesting that an increase in the HCS26/PHO85 kinase activity is required. We have two models for the reason over-expression of HCS26 does suppress, while over-expression of CLN2 does not suppress, the sit4-102 mutant. In model 1, the HCS26/PHO85 kinase can phosphorylate substrates that CLN2/CDC28 cannot. In model 2, the HCS26/PHO85 kinase does not require SIT4 and the CLN2/CDC28 kinase does require SIT4.

The third gene is a previously unidentified gene which we term TAP42 (for two A phosphatase associated protein) because it encodes a 42-kD protein that associates with both SIT4 and the type-2A catalytic subunit (we also isolated TAP42 as a gene that in high copy number suppressed the temperature-sensitive phenotype of a temperature-sensitive pp2a mutant; see below). The TAP42 protein has similarity to the mouse α4 protein, which becomes phosphorylated in response to activation of the T-cell receptor. The budding yeast TAP42 gene is essential, and germinating Δtap42 cells arrest as four to eight unbudded cells. Immunofluorescence microscopy was used to show that TAP42 is enriched in the nucleus, but it is also present in the cytoplasm.

The association of TAP42 with SIT4 does not require the SAP proteins. Moreover, we have not been able to find any TAP42 in SAP immunoprecipitates. These findings show that TAP42/SIT4 complexes are distinct from SAP/SIT4 complexes. In addition, association of TAP42 with PP1H21 or PP1H22 (the type-2A PPase catalytic subunits) does not require TPD3 (an A-like subunit) or CDC55 (a B-like subunit). Together, these findings indicate that TAP42 interacts with the catalytic subunit of SIT4 (a type-2A-related PPase) and with the type-2A catalytic subunits and that this interaction does not require any of the known phosphatase subunits. Moreover, this TAP42 interaction is specific to these phosphatases because TAP42 does not coimmunoprecipitate with the type-1 phosphatase catalytic subunit.

Genetic interactions and in vivo tests indicate that TAP42 functions positively with SIT4 and with the two PP2A catalytic subunits. We propose that the TAP42/phosphatase interactions result in a new activity, which is not present in TAP42 by itself and in SIT4 or the PP2A catalytic subunits by themselves. Possibly, TAP42 might direct the phosphatases to dephosphorylate particular substrates.

Because rapamycin arrests cells in late G₁, and temperature-sensitive sit4 mutants arrest in late G₁, we have been interested in a possible connection between SIT4 and the signaling pathway (TOR1/TOR2) that is inhibited by rapamycin. Interestingly, similar to the TOR mutants that give rapamycin resistance, we have found that certain mutations in tap42 give almost complete resistance to rapamycin. Moreover, the interaction of TAP42 with the phosphatase catalytic subunits is downstream from the TOR1/
TOR2 signaling pathway. Addition of rapamycin to cells rapidly causes the dissociation of TAP42 from SIT4 (but it does not decrease the association of SAP155 or SAP190 with SIT4) and the dissociation of TAP42 from the PP2A catalytic subunits (but it does not dissociate TPD3 [A] and CDC55 [B] subunits from the type-2A catalytic subunit). This effect is not a cell cycle position effect: The cells can be arrested with hydroxyurea or nocodazole, and rapamycin still rapidly dissociates TAP42 from SIT4 and the PP2A catalytic subunits. Therefore, Tor function is actively required to drive the association of TAP with the PPases. Moreover, when the dominant rapamycin-resistant TOR2R gene is present, addition of rapamycin has no effect on the association of TAP42 with SIT4. This suggests that TOR function is needed to cause the association of TAP42 with SIT4 and that the TAP42/SIT4 interaction is a downstream process in TOR signaling. Importantly, TAP42 dissociates from the phosphatases when the cells reach stationary phase. When stationary-phase cells are refed nutrient growth signals (by diluting the cells into fresh medium), TAP42 rapidly associates with SIT4 and with the type-2A catalytic subunits. This association precedes entry into S phase. Importantly, this growth-signal-dependent association of TAP42 with the PPases is prevented by rapamycin. In addition, temperature-sensitive tap42 mutants have a strong defect in the initiation of translation that appears to be identical to that caused by the addition of rapamycin. In addition, like the addition of rapamycin, loss of TAP42 function results in the hyperaccumulation of glycogen. In summary, TAP42/phosphatase complexes are part of the TOR signaling pathway, and their formation is dependent on nutrient growth signals (Fig. 1). Future experiments will be directed at further elucidation of the TAP42/phosphatase interaction for cell cycle progression.

Regulation of the HOG1 Stress-activated MAP Kinase Pathway by TAP42

B. Guo, K.T. Arndt

To understand further how TAP42 functions, we used the yeast 2-hybrid screen to identify TAP42-interacting proteins. From our screen, we isolated 2A phosphatase catalytic subunits (as expected) and two additional genes. One was a novel 41-kDa protein of unknown function whose analysis is in progress. The second TAP42-interacting gene was SSK2, which encodes a MAP kinase kinase kinase for the HOG1 pathway. The HOG1 pathway is required for rapid adaptation to salt stress. Cells lacking HOG1 die when osmotically stressed.

Interestingly, TAP42 interacts with the kinase domain of SSK2. Temperature-sensitive tap42 strains cannot activate the HOG1 MAP kinase at the non-permissive temperature. Moreover, we have isolated tap42 strains that are only salt-sensitive (i.e., they are not temperature-sensitive and they grow normally at 30°C). These strains have a defect in the ability to activate the HOG1 MAP kinase by salt stress. We used the 2-hybrid system to show that one of the salt-sensitive tap42 alleles encodes a TAP42 protein that no longer interacts with SSK2. Therefore, at least one function of TAP42 is to activate the HOG1 MAP kinase via interaction with SSK2. Interestingly, addition of rapamycin rapidly causes the HOG1 MAP kinase to be inactive. Possibly, TAP42/phosphatase complexes link nutrient growth signals to the ability of the HOG1 MAP kinase pathway to be activated by salt stress.

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The human small nuclear RNA (snRNA) gene family contains both RNA polymerase-II-transcribed and RNA polymerase-III-transcribed genes. Yet, all snRNA genes have very similar promoters, suggesting that any difference may be important for the determination of RNA polymerase specificity. The RNA polymerase II snRNA promoters consist of a proximal sequence element or PSE, which is sufficient to direct basal levels of transcription in vitro, and a distal sequence element of DSE, which enhances basal transcription. The RNA polymerase III snRNA promoters contain, in addition, a TATA box located downstream from the PSE, which works in concert with the PSE to direct basal levels of RNA polymerase III transcription. We know that it is the presence of this TATA box that, in the context of the human snRNA promoters, determines RNA polymerase III specificity. Deletion of the TATA box in RNA polymerase III promoters causes a switch to RNA polymerase II transcription, whereas insertion of a TATA box downstream from the PSE in an RNA polymerase II snRNA promoter causes a switch to RNA polymerase III transcription. We are interested in identifying the factors that recognize the snRNA promoter elements and participate in the formation of the initiation complex, and in comparing them with the factors required for RNA polymerase II transcription from mRNA promoters and RNA polymerase III transcription from typical RNA polymerase III promoters with gene internal elements.

The human immunodeficiency virus type 1 (HIV-1) promoter directs the synthesis of two classes of RNA molecules: short transcripts that end prematurely at around position +60, and full-length transcripts. In the absence of the viral trans-activator Tat, most of the RNA molecules derived from the HIV-1 promoter are short, whereas in the presence of Tat, most of them are full-length. The role of the short transcripts is not clear, but several hypotheses have been put forward: In particular, because Tat is an unusual activator that binds to an RNA target contained within the short transcripts, one possibility is that the short transcripts are synthesized to provide binding sites for Tat. Another possibility is that the short transcripts themselves have no role but that the abortive transcription process that produces them serves to keep the HIV-1 promoter in an open position accessible to activators and thus immediately responsive to any transcriptional stimulus. We are interested in understanding how the short transcripts are formed, and what their function is.

Characterization of the SNAP45 Subunit of SNAPc

C.L. Sadowski, R.W. Henry, R. Kobayashi, N. Hernandez

The RNA polymerase II and III snRNA promoters both contain a DSE, which serves as an enhancer of basal transcription. Basal transcription is directed by the PSE in the RNA polymerase II snRNA promoters and by a combination of a PSE and a TATA box in the RNA polymerase III snRNA promoters. The PSE is recognized by a multisubunit complex that we refer to as the SNAP complex (SNAPc). SNAPc is composed of at least four subunits, SNAP43, SNAP45, SNAP50, and SNAP190, and associates with TBP. We have previously isolated a cDNA corresponding to the SNAP43 subunit. We have now isolated cDNAs corresponding to the SNAP45 subunit, as well as to the SNAP50 and SNAP190 subunits (see below). The SNAP45 cDNA encodes a proline-rich 334-amino-acid protein. SNAP45 is specifically co-immunoprecipitated in nondenaturing immunoprecipitations from nuclear extracts performed with anti-SNAP43 antibodies, and reciprocally SNAP45 is specifically coimmunoprecipitated with anti-SNAP45 antibodies. Thus, these two proteins are part of the same complex, although they do not appear to interact directly with each other as determined by GST pull-down experiments. However, both proteins interact directly with TBP.

Immunodepletions of extracts with anti-SNAP45
antibodies inhibit both RNA polymerase II and III transcription of snRNA genes, whereas transcription from the RNA polymerase II adenovirus 2 major late promoter and from the RNA polymerase III VA1 promoter is unaffected. Thus, a SNAP45-containing SNAP complex appears to be required specifically for transcription of genes that contain a PSE, regardless of the RNA polymerase involved.

Characterization of the SNAP50 Subunit of SNAPc

R.W. Henry, B. Ma, C.L. Sadowski, R. Kobayashi, N. Hernandez

We have recently isolated a full-length cDNA clone corresponding to the 50-kD subunit of SNAPc. The open reading frame predicts a 411-amino-acid sequence that contains two putative zinc-binding domains. Depletion of extracts with anti-SNAP50 antibodies inhibits both RNA polymerase II and III transcription of snRNA genes. Together with our previous results, this observation suggests that if two different SNAP complexes are required for RNA polymerase II and III transcription from snRNA promoters, they both contain the SNAP43, SNAP45, and SNAP50 subunits. In-vitro-translated SNAP50 can be coimmunoprecipitated with in-vitro-translated SNAP43, suggesting that these two subunits interact with each other within the SNAP complex. UV crosslinking experiments suggest that SNAP50 contacts the DNA within the SNAP complex. Like recombinant SNAP43 and SNAP45, however, recombinant SNAP50 cannot bind specifically to the PSE, suggesting that the largest SNAPc subunit is the DNA-binding subunit of the complex.

Characterization of the SNAP190 Subunit of SNAPc

M.-W. Wong, R.W. Henry, R. Kobayashi, N. Hernandez

We obtained enough SNAP190 protein for microsequencing and used the protein sequence information to design degenerate oligonucleotides for PCR. We obtained a probe, and by a combination of library screening and PCR, we have isolated a full-length cDNA clone. SNAP190 is the DNA-binding subunit of the SNAP complex, and we are analyzing how this subunit recognizes the PSE.

Characterization of TFIIIB

R. Mital, R. Kobayashi, N. Hernandez

Typical RNA polymerase III promoters with gene internal elements recruit first TFIIIA and/or TFIIIC, which can bind the DNA directly. This in turn allows the recruitment of TFIIIB, which cannot bind to the promoter on its own. TFIIIB is an essential RNA polymerase III transcription factor in that it is thought to contact and recruit RNA polymerase III directly. In yeast, TFIIIB consists of three subunits: TBP and BRF1, which are tightly associated, and the TFC5 gene product, which is more loosely associated with the complex. In mammalian systems, however, the composition of TFIIIB is less clear, although we have shown before that the activity can be split biochemically into two fractions, one of which contains a TBP complex. We have purified the TBP-containing complex and shown that it consists of TBP and one associated protein of 88 kD. The 88-kD protein was subjected to microsequencing, and the protein sequence information was used to design degenerate oligonucleotides for PCR. A specific PCR probe was obtained, which was then used to isolate a partial cDNA clone. A full-length clone was then obtained by 5'RACE. The predicted protein sequence reveals that the 88-kD polypeptide corresponds to the human homolog of yeast BRF1. Human BRF (hBRF) probably corresponds to TFIIIB90, a protein cloned by R.G. Roeder and colleagues, although its predicted amino acid sequence differs over a stretch of 67 amino acids from that reported for TFIIIB90 due to a change of reading frame. Because these 67 amino acids contain matches to two of our peptide sequences, we are confident that our sequence corresponds to the protein we purified.

We raised antipeptide antibodies against peptides derived from various parts of the protein and used these antibodies to examine the role of hBRF, if any, in RNA polymerase III transcription from the human U6 promoter. Some of our earlier results had suggested that the TBP-containing complex contained in the TFIIIB fraction might not be required for U6 transcription. Indeed, we find that depletion of nuclear
extracts with anti-hBRF antibodies inhibited transcription from the VA₁ promoter, a typical RNA polymerase III promoter with gene-internal elements, but had no effect on transcription from the U6 promoter. Furthermore, depletion with a mixture of anti-TBP and anti-hBRF antibodies inhibited RNA polymerase III transcription from both the VA₁ and U6 promoters. However, whereas VA₁ transcription was then restored only by addition of both recombinant TBP and hBRF, transcription from the U6 promoter could be reconstituted to higher than starting levels by addition of just recombinant TBP. Together, these results suggest that the TBP-hBRF complex is not required for U6 transcription. It remains possible that hBRF functions in U6 transcription in a form that is not recognized by our antibodies, for example, as part of another complex. This other complex would then represent, however, a very small percentage of the total hBRF present in nuclear extracts, as the depletions removed more than 98% of the hBRF present in the extract, as judged from immunoblots.

The POU Domain Potentiates the Binding of the SNAP Complex

V. Mittal, M. Cleary, W. Herr, N. Hernandez

The DSE of the RNA polymerase II and III snRNA promoters is characterized by the presence of an octamer motif which can recruit the transcription factor Oct-1. Oct-1 is a POU domain protein whose DNA-binding domain consists of a POU-homeo (POU₄) domain and a POU-specific (POU₅) domain joined by a flexible linker. On a probe containing an octamer motif and a PSE, the Oct-1 POU domain binds cooperatively with SNAP₇. The POU domain of the pituitary transcription factor Pit-1, in contrast, does not have this property. We used this observation to test a number of Oct-1/Pit-1 POU domain chimeric proteins for their ability to recruit SNAP₇ to the PSE. We found that only chimeric proteins containing the amino-terminal part of the Oct-1 POU₅ domain were active. Further mutagenesis showed that exchange of the glutamic acid at position 7 in the Pit-1 POU₅ domain with the glutamic acid present at the corresponding position in the Oct-1 POU₅ domain imparted onto Pit-1 the ability to recruit SNAP₇. The abilities of wild-type and mutant POU domains to enhance snRNA gene transcription in vitro correlated directly with their abilities to recruit SNAP₇ to the PSE. These data suggest that the DNA-binding domain of the Oct-1 activator contributes to transcription activation by recruiting SNAP₇ to low-affinity PSEs.

Characterization of the Cooperative Binding of Oct-1 and SNAP₇ to the DNA

E. Ford, N. Hernandez

The POU domain of the Oct-1 transcription factor can recruit SNAP₇ to the PSE and activate snRNA gene transcription in vitro. Yet, in vivo, Oct-1 domains outside of the POU domain activate snRNA gene transcription. How do these domains function? One possibility is that they simply increase recruitment of SNAP₇ by the POU domain. To test this possibility, we compared full-length Oct-1 and the Oct-1 POU domain for their ability to recruit SNAP₇ to the PSE. Although full-length Oct-1 was slightly more active than the POU domain, the difference was small and is therefore unlikely to account for the effect observed in vivo. This suggests that activation by Oct-1 involves at least two steps: recruitment of SNAP₇ to the PSE, and another step performed by Oct-1 domains outside of the POU domain.

We have characterized a PSE-binding complex present in nuclear extracts, which we can detect by electrophoretic mobility shift assay. This complex contains both SNAP₇ and Oct-1, even though Oct-1 on its own cannot bind to the probe. Thus, SNAP₇ can recruit Oct-1 to a probe which does not contain an Oct-1-binding site. Nevertheless, we could not detect binding of Oct-1 to SNAP₇ in the absence of DNA. We therefore determined whether Oct-1 contacts DNA in the complex. Indeed, Oct-1 contacts an ATT sequence present on the probe. ATT (or AAT on the other strand) constitutes part of the AAAT sequence recognized by the POU₄ domain on a histone H2B octamer site, suggesting that it may be contacted by the POU₄ domain.
TBP and SNAPc Bind Cooperatively to the Human Basal U6 Promoter
V. Mittal, N. Hernandez

The basal U6 promoter consists of a PSE and a TATA box located at a fixed distance downstream from the PSE. The PSE recruits SNAPc, whereas the TATA box recruits the TATA-box-binding protein TBP. TBP has been cloned from a number of species, and sequence comparison reveals that the protein can be divided into two domains: A very highly conserved carboxy-terminal domain, and a nonconserved amino-terminal domain. The carboxy-terminal domain performs all the functions so far attributed to full-length TBP, including binding to the TATA box, interacting with other basal transcription factors and activators, and directing basal RNA polymerase II transcription in vitro.

We have examined whether SNAPc and TBP might bind cooperatively to their respective binding sites in the U6 promoter. We find that this is indeed the case and that cooperative binding requires the amino-terminal domain of TBP. This suggests that one of the roles of the nonconserved amino-terminal domain of TBP is in snRNA gene transcription by RNA polymerase III.

Factors Required for snRNA Transcription by RNA Polymerase II
T.L. Calhoun, N. Hernandez

The RNA polymerase II snRNA promoters require the SNAP complex for transcription, but little is known about which other factors are required. In contrast, all of the factors required for basal transcription from the adenovirus 2 major late promoter, a typical RNA polymerase II mRNA promoter, have been identified, purified, and in most cases cloned. In collaboration with Dr. D. Reinberg and colleagues, we are determining which of the general transcription factors required for transcription from the adenovirus 2 major late promoter are also required for transcription from RNA polymerase II snRNA promoters. We are using antibodies directed against various general transcription factors to deplete extracts and test whether RNA polymerase II snRNA transcription is affected. In the cases where it is, we then ask whether addition of the recombinant factor can reconstitute transcription. These experiments will provide an insight as to which factors direct RNA polymerase II transcription from snRNA promoters.

Identification and Purification of FBI-1, a Factor That Specifically Recognizes the IST
F.C. Pessler, P.S. Pendergrast, N. Hernandez

We have previously shown that formation of short transcripts from the HIV-1 promoter depends on a DNA element located in large part downstream from the HIV-1 transcription start site. This element, referred to as IST for "inducer of short transcripts" is bipartite, with the 5' module being the most important for IST activity. The biochemical role of IST is not known, but we imagine that IST enhances the recruitment of RNA polymerases that are not capable of efficient elongation. Because IST is active in the
absence of any viral proteins, it must function by recruiting a cellular factor. We have identified an IST-binding factor (FBI-1) whose binding to wild type and mutated versions of the IST correlates well with short transcript formation. FBI-1 is therefore a good candidate for a factor involved in the synthesis of short transcripts. FBI-1 has been purified to near homogeneity and consists of a single polypeptide whose size is identical to that of a polypeptide that can be specifically cross-linked to the IST in partially purified fractions. This factor binds to several cellular promoters that are known to be regulated at the level of elongation and may therefore play a part in abortive transcription from a number of cellular promoters.

Isolation of cDNAs Corresponding to FBI-1

D.J. Morrison, P.S. Pendergrast, R. Kobayashi, N. Hernandez

As a first step toward the understanding of the role of FBI-1, we have scaled up the purification of FBI-1 and obtained enough protein for microsequencing. Searches of the expressed sequence tag (EST) database revealed one EST whose predicted amino acid sequence contained two of the peptide sequences obtained from FBI-1. We used the EST as a probe to screen a λ library and obtained a cDNA that encodes a protein comigrating with endogenous FBI-1. This suggests that the cDNA might be full-length. We have raised antipeptide antibodies and are using these reagents in an effort to understand the biochemical function of FBI-1.

TBP Domains Required for Tat Trans-activation and IST Function

P.S. Pendergrast, D.J. Morrison, W.P Tansey, M. Sheldon, N. Hernandez

The HIV-1 promoter generates two types of RNA molecules, full-length transcripts and short transcripts. The synthesis of the short transcripts depends on the IST element. In the presence of the viral trans-activator Tat, the synthesis of full-length transcripts is up-regulated, whereas that of the short transcripts is down-regulated. Thus, although IST appears to stimulate the formation of transcription complexes that are incapable of efficient elongation, Tat appears to stimulate the formation of transcription complexes that are capable of efficient elongation.

The HIV-1 promoter contains a TATA box. We have used an in vivo assay to assess the role of TBP in HIV-1 transcription and to test the effects of mutations in TBP on synthesis of both short and full-length transcripts. We found that TBP bound to the TATA box is required for the synthesis of short and full-length transcripts as well as for Tat trans-activation. Both the carboxy-terminal domain of human TBP and full-length yeast TBP can replace full-length human TBP for all of these processes. Mutations in TBP affect formation of short and long transcripts as well as Tat activation similarly, and these effects correlate with the previously described effects of these mutations on binding of TBP to the TBP-associated factor TAF1250. These results suggest that although IST and Tat may stimulate the formation of different types of transcription complexes, they use TBP similarly, most probably as part of the TFIID complex.

Comparison of RNA-targeted and DNA-targeted Transcriptional Activators

P.S. Pendergrast, N. Hernandez

Tat is unique among transcription activators in that it acts through an RNA target, the TAR element, contained within the short transcripts. However, when fused to the GAL4 DNA-binding domain, Tat can also activate transcription from GAL4-binding sites on the DNA, raising the question as to why Tat is directed to the RNA. We have compared RNA-targeted activators and DNA-targeted activators in their ability to activate the synthesis of full-length transcripts and to repress the synthesis of short transcripts from the HIV-1 promoter. We find that DNA-targeted activators including DNA-targeted Tat activate the synthesis of both short and long transcripts, whereas the RNA-targeted activators Tat and a Rev-VP16 fusion protein activate the synthesis of full-length transcripts but repress the short transcripts. Thus, repression of the short transcripts seems to be a property unique to RNA-targeted activators, suggest-
ing that repression of the short transcripts may be an important function of Tat.

PUBLICATIONS


In Press


TELOMERASE BIOCHEMISTRY AND REGULATION


Telomerase is a ribonucleoprotein polymerase that synthesizes telomere repeats onto chromosome ends. Evidence from yeast and mammalian cell culture suggests that telomerase is required for telomerase length maintenance. Telomerase activity is often not detected in human primary cells and tissues, whereas tumor-derived cell lines and many tumor samples contain detectable telomerase activity. The presence of telomerase activity and short telomeres in tumors suggested that inhibiting telomerase may be an effective cancer treatment. To understand both the biochemistry of this unusual enzyme and the role of telomerase in cancer, we are studying telomerase from Tetrahymena, human, and mouse. In the past year, we have extended our in vitro studies of reconstituted Tetrahymena telomerase to human telomerase to determine the essential regions of the human telomerase RNA. We have also begun to dissect the pathways that regulate telomerase in human cells through understanding the cell cycle regulation in white blood cells.

Finally, in collaboration with Dr. Ronald DePinho, we generated a mouse that lacks telomerase activity. This telomerase "knockout" mouse provides an essential tool to study the role of telomerase in mouse development and in cancer.
Human Telomerase Reconstitution
C. Autexier, R. Pruzan [in collaboration with W. Funk, Geron Corporation]

To dissect the human telomerase enzyme mechanism, we developed a functional in vitro reconstitution assay. After removal of the essential 445-nucleotide human telomerase RNA (hTR) by micrococcal nuclease digestion of partially purified human telomerase, the addition of in-vitro-transcribed hTR reconstituted telomerase activity. The activity was dependent on and specific to hTR. Mutations in the hTR template region generated the expected altered telomere sequence repeats in vitro. Using this assay, truncations at the 5′ and 3′ ends of hTR identified a minimal functional region of hTR (Fig. 1). Surprisingly, we found that residues 1–44, 5′ to the template region, were not essential for activity, indicating that a minimal functional region is located between residues 44 and 203. Mutagenesis of full-length hTR between residues 170 and 179, 180 and 189, or 190 and 199 almost completely abolished the ability of the hTR to function in the reconstitution of telomerase activity, suggesting that sequences or structures within this 30-nucleotide region are required for activity, perhaps by binding telomerase protein components.

Telomerase Regulation during Entry into the Cell Cycle
K. Buchkovich

Leukocytes, unlike many human somatic tissues, have detectable telomerase activity. These cells provided a normal human cell type in which to study telomerase. We studied the regulation of telomerase activity and the telomerase RNA component as leukocytes were stimulated to enter the cell cycle. In primary human leukocytes stimulated with phyto-
hemagglutinin, telomerase activity increased greater than tenfold as naturally quiescent cells entered the cell cycle. The timing of the increase in telomerase activity correlated with the increase in the percentage of cells in S phase. Antibodies to the T-cell receptor (TCR)/CD3 complex and the costimulatory CD28 receptor induced telomerase activity in a T-cell-enriched population of cells. Rapamycin, an immunosuppressant that blocks TCR/CD3 signal transduction pathways and Cdk2 activation, blocked telomerase induction. Hydroxyurea, an inhibitor of S phase, did not block Cdk2 kinase activity or telomerase activation. Analysis of several cell cycle markers including Cdk2 kinase activity and cyclin E, and p27Kip1 by Western blotting, indicated that rapamycin inhibited cells in G1 phase and hydroxyurea blocking cells in early S phase, indicating that telomerase is activated in G1 phase as T cells enter the cell cycle.

**Generation of a Telomerase "Knockout Mouse"**

M. Blasco [in collaboration with Han-Woong Lee and Ronald DePinho, Albert Einstein College of Medicine]

To test whether telomerase is required for viability and for tumor formation, we used homologous recombination to generate mice that were deleted for the mouse telomerase RNA gene (mTR). Heterozygous mice were initially generated that had one allele of mTR replaced by the neomycin gene. The offspring from crossing mTR+/− together showed a 1:2:1 Mendelian distribution of wild-type (mTR+/+), heterozygous (mTR+/−), and null mice (mTR/−/−), indicating that the mouse telomerase RNA gene is not essential for mouse development. To determine whether the absence of mTR affects fertility, first-generation (G1) mTR/−/− mice were crossed to each other and second-generation (G2) mTR/−/− mice were obtained in the expected numbers. Further mTR/−/− intercrosses generated third (G3), fourth (G4), fifth (G5), and sixth (G6) generation null mice, indicating that mTR is not essential for mouse development or germ-line function.

Tissues and cell lines derived from telomerase null mice lacked telomerase activity as measured by two independent assays (Fig. 2). The absence of telomerase activity in mTR/−/− mouse embryo fibroblasts (MEFs) and tissues indicates that the mTR gene is essential for mouse telomerase activity in vivo. To determine if mTR/−/− cells retain the ability to express telomerase activity, we reintroduced the wild-type gene into mTR/−/− MEF cultures by transient transfection. Cell cultures transfected with the wild-type gene had detectable telomerase activity, whereas cultures transfected with an empty vector had no detectable telomerase activity (Fig. 2). This in vivo reconstitution of telomerase indicates that the telomerase RNA is the only component the cells lack for the expression of telomerase activity.

**GENERATION OF ESTABLISHED CELL LINES FROM MTR/−/− MICE**

To address the question of whether telomerase has a role in cell immortalization and tumor formation, we studied the ability of telomerase-negative (mTR/−/−) and telomerase-positive (mTR+/+) MEFs to generate established cells lines using the 3T3 protocol. MEF cultures from the first-generation knockout (G1) and mTR/−/− cultures from G2, G3, and G4 generations of knockout mice had growth rates similar to those of the wild-type mTR+/+ cells for more than 200 generations. The telomerase null cultures generated established cell lines after a period of slow growth known as senescence or crisis at rates similar to those of wild-type cells. This indicates that telomerase activity is not required in mouse cells to escape senescence or for the establishment of permanent cell cultures.

**TRANSFORMATION AND TUMOR FORMATION IN THE ABSENCE OF TELOMERASE ACTIVITY**

To study the role of telomerase activation in mouse tumors, we tested both primary and established mTR/−/− and mTR+/+ cells for oncogenic transformation and tumor formation with SV40 T antigen plus rasv12. Foci from transfromed mTR/−/− and mTR+/+ cells had a similar morphology and growth rate. When injected into two different sites on a nude mouse, the mTR/−/− and mTR+/+ transformed cell lines generated tumors of similar size. To look at the effect of long-term in vivo absence of telomerase, early-passage MEF cultures from G1 mTR+/+, and mTR+/− and G1, G2, G3, and G4 mTR/−/− mice were transformed with adenovirus E1A and rasv12. Foci were selected and 10⁵ cells were injected into nude mice. Tumors were generated from all of the mTR/−/− G1, G2, G3, and G4 cells (Fig. 3). Although there was significant scatter in tumor size, there was no correlation with the genotype or generation of mTR/−/−.
animals (Fig. 3). These results indicate that telomerase activity is not essential for tumor formation in nude mice.

TELOMERE LENGTH IN TELOMERASE NULL CELLS

Telomerase activity is thought to be required for telomere length maintenance in mammals. In human cells, the terminal restriction fragment (TRF) containing the telomeric repeats is typically between 10 and 15 kb. However, the mouse laboratory species, Mus musculus, used to generate the mTR−/− strain, has telomeres ranging from 20 to 150 kb. The TRFs are not resolved on conventional agarose gels and thus pulsed-field gels have been used to estimate TRF length. Surprisingly, using pulsed-field gels, we were not able to detect significant telomere shortening in mTR−/− MEFs compared to mTR+/+ cells after growing the cells for more than 200 population doublings in vitro. Furthermore, primary MEFs from mTR+/+ and mTR−/− from the G1, G2, G3, and G4 generations did not show any significant difference in telomere length when compared to wild-type mTR+/+ DNA (Fig. 4). The inability to detect telomere shortening suggests that the rate of telomere shortening in the absence of telomerase activity in M. musculus might be slower than in human cells, and further passaging will be needed to detect telomere shortening. Alternatively, the apparent lack of telomere shortening in mouse cells might reflect a telomerase-independent mechanism for telomere maintenance. Yeast cells deleted for the telomerase RNA gene that have a wild-type recombination system generate rare "survivors" after loss of viability in culture. In these cells, gene conversion allows lengthening of telomere repeat tracts. If the rad52 gene is deleted, gene conversion is severely reduced and survivors are not generated. In yeast undergoing recombination-mediated telomere elongation, dramatic changes in telomere length are seen. Strikingly, even in wild-type mice, the rate of telomere length changes is extraordinarily high, suggesting that perhaps recombination pathways may normally have a role in telomere maintenance in the mouse.

RELEVANCE TO HUMAN CANCER

Although mice provide a powerful tool for understanding cancer progression, there are some sig-
significant differences in human and mouse biology that may effect tumor growth. Two of the most relevant differences for this study are the extremely long telomeres in mice and the relative ease of immortalization of mouse cells. Telomerase activation is tightly correlated with immortalization in human cells, whereas mouse cells immortalized readily in the absence of telomerase. Whether this difference represents a fundamentally different tumor suppressor mechanism in human cells or is simply a slight difference in control pathways remains to be established.

The possibility that mouse cells easily activate or perhaps normally utilize a telomerase-independent mechanism of telomere maintenance is intriguing. Evidence suggests that recombinational bypass pathways might occur in some human cell lines. Since this alternative mechanism might provide an avenue for human tumor survival, it will be essential to fully understand the role of this pathway in both human and mouse cells.

The fact that a mouse lacking telomerase can survive for more than six generations and the growth rate of cells in vitro is not altered was surprising to us and to most of our colleagues. However, unexpected results are often the most interesting as they allow us to challenge our own assumptions and to refocus on what our experiments are telling us about nature.

**PUBLICATIONS**


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**PLANT GENETICS**

V. Sundaresan  
R. Martienssen  
E. Grotewold

W. Flood  
T. Litzi (URP)  
G. Moriates  
M. Pizziruoso  
P.D. Rabinowitz  
Y. Sun  
D. Bush

M. Byrne  
M. Curtis  
Q. Gu  
J. Montagu  
A.M. Settles  
P. Stavropoulos

N. Springer (URP)  
P. Springer  
C. Yordan  
J. Colasanti  
S.T. Woody  
Z. Yuan

**Molecular Analysis of the Maize Indeterminate Gene**

J. Colasanti, Z. Yuan, V. Sundaresan

**THE MAIZE INDETERMINATE GENE REGULATES THE TRANSITION TO FLOWERING**

Plants differ from animals in that most of their development occurs postembryonically. This is accomplished by a population of undifferentiated stem cells, the apical meristem, which gives rise to all parts of the plant after germination. The shoot apical meristem generates the aerial parts of the plant. During vegetative growth, the shoot apical meristem produces leaves and other vegetative structures, and during reproductive growth, the shoot apical meristem gives rise to structures such as flowers. A critical and poorly understood event in the development of plants occurs when the shoot apical meristem undergoes the transition to reproductive growth. We have been studying a mutant of maize, *indeterminate* (*idl*) that is defective in the transition to reproductive develop-
ment, i.e., the shoot apical meristem of id1 mutants continues to produce leaves, therefore maintaining the plant in a state of vegetative growth. Eventually, given enough time, id1 mutants will produce aberrant floral structures with vegetative characteristics. Previously, we reported the isolation of the id1 gene from maize by transposon tagging. This year, we report the complete structure of the id1 gene and describe the expression pattern of id1 during development.

THE ID1 GENE STRUCTURE

The sequence of the id1 gene shows that it has similarities to zinc finger regulatory proteins from other species, suggesting that id1 may act as a transcriptional regulator of other genes. The structure of the id1 transcribed region was determined by RT-PCR. Primers based on the sequence of an id1 genomic clone were used to amplify portions of an id1 transcribed regions. Comparison of the amplified regions to the genomic sequence showed that id1 has three introns and four exons (see Fig. 1). Exons 2 and 3 each contain a zinc finger DNA-binding motif; the other parts of the deduced amino acid sequence show no similarity to proteins in current databases. The zinc finger motif domain, however, shows homology with the Drosophila and human Kruppel genes and was used to identify a family of genes in maize with this motif. These have been designated id-like genes. In addition, id-like genes have been identified in other plant species, such as Arabidopsis and potato. We are currently collaborating with Pioneer Hi-Bred International to investigate the function of the id-like genes.

THE ID1 GENE IS EXPRESSED IN DEVELOPING LEAVES

Northern blot analysis was used to determine where

id1 is expressed in the plant. Poly(A)* mRNA was isolated from different parts of maize plants at different times of growth, from soon after germination to late in development when flowers have already formed. Spatially, the id1 gene is expressed only in immature leaf tissue that has not expanded and is not undergoing photosynthesis; i.e., it is absent in mature leaves. Temporally, we have discovered that id1 is expressed at low levels soon after germination and that expression increases as plants approach the transition to flowering. After formation of the flowers, the id1 mRNA is detectable at low levels in post-reproductive plants. Expression of id1 is not found in roots, stems, or floral tissues. More importantly, Northern analysis and preliminary in situ hybridization experiments show no detectable levels of id1 in the shoot apical meristem at any time during development (see Fig. 2). This has significant implications for the mechanism of id1 gene function (see below).

id1 REGULATES A TRANSMISSIBLE SIGNAL THAT EVOKES THE TRANSITION TO FLOWERING

A large number of physiological studies from the last 70 years have demonstrated that two critical events must occur for a plant to undergo the transition to flowering: First, a signal is produced in the leaves that is transmitted to the shoot apical meristem where it elicits the flowering program, and second, the shoot apical meristem must become competent to receive the floral stimulus. Although many attempts have been made to isolate a flower-inducing substance (or "florigen"), no one has succeeded in identifying a simple hormone-like substance that can cause the cells of the shoot apical meristem to begin a program of reproductive development. As reported in previous Annual Reports, we have genetic evidence that id1 acts non-cell-autonomously, i.e., that the id1 gene product mediates the production of a signal that
migrates from one part of the plant to another to carry out its function. The expression pattern of idl described above now provides molecular evidence that the idl gene acts non-cell-autonomously, i.e., that it is expressed in leaves where it regulates the production of a substance(s) that migrates to the shoot apical meristem to mediate the transition from vegetative to reproductive growth. Current efforts are directed at pin-pointing the temporal/spatial expression pattern of the idl gene and at examining the location of the ID1 protein. Understanding the molecular signals that invoke reproductive development will contribute to the understanding of how this process occurs in plants, as well as have potential commercial benefits from the ability to manipulate reproductive timing in crop plants.

Genetic Analysis of Embryogenesis in Arabidopsis Using Enhancer Trap and Gene Trap Transposon Tagging

S.T. Woody, V. Sundaresan

We have used the enhancer trap and gene trap transposon tagging system developed at Cold Spring Harbor Laboratory (Sundaresan et al., Genes Dev. 9: 1797 [1995]) to identify and characterize embryo-specific gene expression in Arabidopsis. Nearly 1000 insertion lines generated by our laboratory and the collaborating laboratories of Grossniklaus, Ma, and Martienssen here at the Laboratory were screened for patterns of GUS reporter gene expression during embryogenesis. Three lines that displayed an embryo-

FIGURE 2 Northern blot analysis with mRNA from mature leaf, immature leaf, root, and apex from B73 maize plant with ten visible leaves. The same blot was probed with an idl-specific probe, the maize cdc2 probe (which is highly expressed in tissues with actively dividing cells), the hcf106 gene probe (expressed in leaves), and a probe for an id-like gene, "p1".
specific GUS expression pattern are of particular interest. In LENNY, GUS expression is first detected in the lens-shaped cell of the hypophysis at the base of globular-stage embryos and remains confined to it throughout embryogenesis. In the mature embryo, staining is present only in the central cells of the root meristem. LENNY marks a specific cell lineage within the basal domain of the embryo and provides separate confirmation of the proposal that the central cells are descendants of the lens-shaped hypophysial cell (Dolan et al., Development 119: 71 [1993]). In contrast, the expression specificity of COTDOT is dynamic: Expression is first observed in cells near the nascent shoot apical meristem of transition stage embryos, but at later stages, GUS expression becomes more localized, becoming confined to vascular cells of the embryonic root and ultimately to a subset of cells in the root meristem and columella. Finally, expression in a third insertion, JART, occurs in the specialization zone of the root; expression is also observed in the cotyledons. It was determined that the insertional Ds element in JART is within an exon downstream from the homeodomain of a member of the GLABRA2 family of Arabidopsis homeobox proteins. More detailed characterization of the above three insertions and other insertion lines identified in this screen is in progress.

Gene Trap Patterns during Organogenesis in Arabidopsis

P. Springer, Q. Gu, D. Bush, R. Martienssen

The molecular mechanisms underlying the partitioning, outgrowth, and differentiation of lateral organs in plants are largely unknown, but leaves, floral organs, and cotyledons (seed leaves) may use the same basic process, given that these organs can be interconverted by homeotic mutations. Because of its highly reiterative nature, leaf development is uniquely suited to study organogenesis in plants. Unfortunately, many mutations in genes required for leaf development result in lethality or sterility because of their effects on leaf-like organs in the embryo and in the flower. This makes such mutations difficult or impossible to identify in conventional genetic screens. We are using enhancer trap and gene trap transposon mutagenesis in the model plant Arabidopsis thaliana to identify genes expressed in prepatterns in meristems and leaf primordia (Sundaresan et al., Genes Dev. 9: 1797 [1995]). Using this approach, genes can be identified by their expression patterns in heterozygous (normal) plants even when disruption results in an early lethal phenotype (Springer et al., Science 268: 877 [1995]). We have screened 1034 gene trap lines and 880 enhancer trap lines for reporter gene expression in the seedling 9 days after germination and have established a database containing these expression patterns. Approximately 45% of enhancer traps (397) and 31% of gene traps (328) show GUS expression. Figure 3 shows the number of expression patterns that are specific to each individual organ with respect to any two other organs. Ubiquitous expression patterns are rather rare, representing about 6% of the total (43/725), and many of these are artifactual insertions in the T DNA. Approximately 4% of the lines show reporter gene expression exclusively in leaves or leaf primordia, whereas 4.5% exhibit GUS expression specifically in other domains of the shoot apex. Amplification and sequencing of transposon insertion sites has revealed insertions into
a wide variety of genes. Insertions into two known genes (the MADS box gene AGL8 and the ethylene response gene hookless1) have reporter gene expression patterns that are in close agreement with published in situ hybridization data. We believe therefore that this collection accurately reflects the proportion of all expressed genes that are found in these tissues. By extrapolation, several hundred genes may be specifically expressed in prepatterns in the shoot apex.

Molecular Genetics of asymmetric leaves in Arabidopsis

M. Curtis, R. Martienssen

The Arabidopsis mutant asymmetric rosette leaves (asl) has variably lobed leaves with a drastically altered proximodistal pattern, characterized by altered spacing of hydathodes, trichomes, and veins relative to the leaf margin. We are examining the interaction of asl with several GT and ET lines which have reporter gene expression patterns in the shoot apical meristem and the leaf primordia. We have identified several genes whose expression pattern is altered in asl mutants, and an example is shown in Figure 4A. Similarly, ET and GT lines with expression patterns in the stomata, the vasculature, stipules, hydathodes, trichomes, and marginal epidermal cells are being used to examine the position of these landmark structures in asl leaves. We are attempting to clone the gene for asl using the gene-trap transposon system. Ds transposable elements preferentially transpose within 5 cM of their original location, and one of the Ds starter lines (DsG1) has been mapped to chromosome 2 less than 5 cM away from asl (Sundaresan et al., Genes Dev. 9: 1797 [1995]). Approximately 40,000 F1 seeds derived from more than 1500 Ac DsG1 X asl crosses have been produced. Plants derived from these F1 seeds are currently being screened for new alleles of asl, caused by the insertion of Ds, which will allow molecular cloning of the locus. In parallel experiments, we have obtained 135 recombinants around the asl locus that have allowed us to map asl to a single YAC. Complementation of mutant plants with cosmid clones from this region are currently under way using Agrobacterium-mediated transformation.

Disruption of the AGL8 Gene Prohibits Differentiation during Arabidopsis Fruit Development

Q. Gu, R. Martienssen [in collaboration with C. Ferrandiz and M. Yanofsky, Department of Biology and Center for Molecular Genetics, University of California at San Diego]

We have isolated an enhancer trap DsE insertion into the 5'-untranslated region of the MADS box gene AGL8 (agamous-like). GUS reporter gene expression is found in the vascular tissue of the leaf and stem. In the gynoecium, GUS expression is confined to all cell layers of the carpel valve but is excluded from the dehiscence zone, the replum, and septum tissues. Expression of the AGL8-coding region is abolished in the mutant. The Arabidopsis fruit (the silique) is derived from two fused carpels, which greatly elongate after fertilization. Dehiscence takes place at ripening to release the seeds. Plants homozygous for the agl8 mutation have small congested siliques. Instead of an orderly dehiscence, seeds are trapped in the silique and occasionally they prematurely burst through the carpel wall. In addition, the cauline leaf is noticeably broader as compared to that of the wild type. The small carpel structure is due to a failure of cellular differentiation after fertilization. Scanning microscopy showed that several cell types are missing, including stomatal cells. Cell division and expansion are disrupted in the carpel wall but continue in the central septum tissue of the silique. Although the only phenotype we can detect is in the fruit, expression at other stages of development suggests that AGL8 may have a redundant function in these other tissues. This possibility is being addressed in double-mutant studies.

FIGURE 4 Enhancer trap and gene trap reporter gene expression patterns. An enhancer trap reporter gene expression pattern at the base of the wild-type leaf (A) is severely reduced at the base of asymmetric leaves at the same stage (B). (C) An enhancer trap expression pattern confined to leaf vascular tissues.
Characterization of the Prolifera Gene in Arabidopsis
P. Springer, R. Martienssen

We have previously reported the cloning and characterization of the PROLIFERA gene from Arabidopsis (Springer et al., Science 268: 877 [1995]). PROLIFERA was identified by a DsG (gene trap Ds element) insertion, which led to reporter gene expression in dividing cells. PROLIFERA is closely related to the CDC47 gene from Saccharomyces cerevisiae, which is a member of the MCM2-3-5 gene family, and is required for the initiation of DNA replication. Consistent with this predicted essential function, disruption of PROLIFERA by the DsG element results in megagametophyte and embryo lethality. This early lethality masks any role PROLIFERA may have at later stages of development. We have begun experiments which will allow us to examine the role of Prolifera throughout development. We have made constructs containing a DsG element fused between a strong constitutive promoter (CaMV 35S) and the PROLIFERA gene in either the sense or antisense orientation. In the absence of Ac, plants carrying one of these constructs will express the GUS reporter gene constitutively, under control of the 35S promoter. In the presence of Ac, the DsG element will excise in somatic sectors, resulting in loss of GUS activity and the concomitant expression of the Prolifera or anti-Prolifera gene. This will provide a visual marker (loss of GUS activity) for the antisense or overexpression sectors. In this way, we can examine whether cellular differentiation depends on cell cycle progression in the developing leaf and root.

Using Gene Trap and Enhancer Trap Screens to Identify Gene Products That Regulate Resource Allocation in the Plant's Vascular System
D. Bush (1), R. Martienssen

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The leaf functions as the primary supplier of free energy and reduced carbon in higher plants. The leaf is the so-called "source" tissue because it contains the photosynthetic machinery that transforms light energy into useful biological energy and because most of that captured energy is exported from the leaf (in the form of sucrose and amino acids) to satisfy the biochemical needs of nonphotosynthetic cells. Likewise, the import-dependent tissues specialize in other important processes, such as nutrient acquisition (roots) or reproduction (flowers). The development of specialized tissue systems generates a need for long distance transport, and the vascular system of higher plants is the complex network of specialized cells that mediate this essential process. Several gene trap and enhancer trap transposants have been identified whose GUS expression pattern is restricted to the vascular system in mature leaf tissue (Fig. 4B). Since the principal activity of mature leaves is the acquisition and export of sugars and amino acids, the protein products encoded by these tagged genes may be important contributors or regulators of this fundamental activity. Significantly, several of these transposants exhibit mutant phenotypes that include reduced size and slow growth. In addition to leaf expression patterns, other transposants have been identified whose GUS expression is localized in the vascular tissue of the hypocotyl and/or roots of the plant. Current efforts in this project are focused on obtaining sequence information about the tagged genes and on using thin sections of stained organs to identify the cell-specific expression patterns.

Local Saturation Mutagenesis of the Short Arm of Arabidopsis Chromosome 4
C. Yordan, J. Montagu, P. Slavopolous, R. Martienssen, [in collaboration with M. Lodhi and R. McCombie, Cold Spring Harbor Laboratory]

The Cold Spring Harbor Arabidopsis sequencing consortium will be determining the complete nucleotide sequence of the short arm of chromosome 4 (a 3-Mb region) during the next 2–3 years (see report by R. McCombie). We are attempting to saturate this region with transposon insertions launched from the prolifera gene, which lies near the center of this region. By isolating DNA in pools from a collection of such insertions, we can identify insertions into the sequenced genes by amplifying pooled DNA with primers from the sequence and primers from the DsG transposable element (Das and Martienssen, Plant Cell 7: 287 [1995]). Mobilization is accomplished by
crosses to Ac transposase, followed by selection of pooled F₂ progeny on culture plates supplemented with naphthalene acetamide (NAM) which selects against Ac, and kanamycin, which selects for the Ds, either at prolifera or at a new location. We have analyzed a few hundred selections by Southern blotting, amplification, and mapping against the YAC contig from this region. Results suggest that insertion frequencies are high enough for this procedure to be effective and that insertions typically fall within a few hundred kilobases of the prolifera gene. Nevertheless, many thousands of selections need to be made to saturate the region. A number of insertions result in new patterns of reporter gene expression and in mutant phenotypes, which are under investigation.

The iojap Gene in Maize

M. Byrne, R. Martienssen

iojap (ij) is a recessive nuclear mutation in maize that affects the differentiation of mature chloroplasts from undifferentiated organelles. The cytoplasmic inheritance of defective plastids from variegated ij plants has been a textbook example of non-Mendelian genetics for more than 50 years (Rhoades, Cold Spring Harbor Symp. Quant. Biol. 11: 202 [1946]). Molecular cloning and preliminary biochemical studies suggest that the maize Ij protein is associated with the 50S subunit of chloroplast ribosomes (C.-D. Han and R. Martienssen, Annual Report 1994). Hypothetical proteins with sequence similarities to Ij have been identified in a number of other organisms, including Arabidopsis (M. Byrne and W. Taylor, unpubl.). The maize and Arabidopsis sequences are 64% identical except for an amino-terminal region that is predicted to be a transit peptide cleaved during chloroplast import. Hypothetical proteins from five bacterial species share between 23-36% identity and 50-57% similarity with the maize Ij. In addition, a hypothetical protein in Caenorhabditis elegans shares 21% identity (50% similarity) with maize Ij and has all of the amino acid residues absolutely conserved in the above sequences. The C. elegans protein has an amino-terminal extension suggestive of a mitochondrial transit peptide. However, there is no obvious Ij homolog in yeast. Bacterial overexpression of the mature protein from maize and Arabidopsis has been accomplished using His-tagged proteins, antibodies have been raised, and yeast 2-hybrid screens are being used to identify interacting proteins.

Genetically, double mutants have been constructed with other plastid ribosome mutants, and a number of new alleles of maize Ij have been generated using the transposon Mutator. The phenotype of homozygous ij-mum17 plants is dependent on the activity of Mu transposons, so that homozygous plants that have lost Mu activity appear wild type. This suppressible ij allele will provide a useful tool for cell lineage studies because double mutants for ij-mum17 and the pigment marker al-mum2 will be coordinately mosaic for both genes in clonally derived sectors (Martienssen and Baron, Genetics 136: 1157 [1994]). The effect of loss of Ij function can then be assessed in sectors that are marked by loss of pigmentation.

Specification of Leaf Shape and Leaf Cell Identity in leafbladeless2 Mutants of Maize

N. Springer, R. Martienssen [in collaboration with M. Timmermans and T. Nelson, Yale University]

leafbladeless2 is a recessive leaf mutant that arose in a Robertson's Mutator line. The mutant phenotype is suppressed in Mu-inactive stocks and in mosaic plants, suggesting it was caused by the insertion of a Mu transposable element. The first two leaves have wild-type morphology, but in successive leaves, the width and pigmentation of the blade becomes severely reduced until only a pale thread remains. Morphological defects could be observed in very early primordia by SEM, suggesting that fewer cells are incorporated into mutant leaf primordia. In leafbladeless2 mutants, ectopic ligule will often form on the abaxial surface of the leaf at the sheath-blade boundary. Ectopic sheath fringes are also observed in some plants, running parallel to the sheath margin, and flanking patches of adaxial epidermal cell types on the abaxial side. Residual mutant blade tissue has fewer mesophyll cells and almost no intermediate bundles. Lateral vascular bundles exhibit a chaotic placement in the horizontal axis but appear to retain wild-type polarity in the dorsoventral axis. The pleiotropic phenotype suggests that Leafbladeless 2 is required for leaf cells to adopt the appropriate identity in response to axis specification in the early primordia. It remains to be seen whether this reflects an alteration in axis specification itself.
Hcf106 is an Ancient Conserved Gene Required for Membrane Protein Translocation in Chloroplasts

A.M. Settles, D. Bush, R. Martienssen
[in collaboration with K. Cline, University of Florida, Gainesville]

Hcf106 mutant maize seedlings are pale green and are deficient in a subset of chloroplast thylakoid membrane protein complexes. Among other possibilities, a defect in thylakoid protein targeting could lead to the observed phenotype. Thylakoid protein targeting in chloroplasts utilizes translocation pathways found in bacteria, via the homologous receptors cpSecA and cpFfh (Cline and Henry, Annu. Rev. Cell Dev. Biol. 12: 1 [1996]). In addition, a subset of thylakoid proteins are targeted using a third pathway that requires only a pH gradient across the thylakoid membrane (the ΔPH pathway). hcf106 mutants accumulate intermediates of known substrates for this targeting pathway, suggesting that it may be defective (Voelker and Barkan, EMBO J. 14: 3905 [1995]; Settles et al., CSHL Annual Report 1994). We have adapted in vitro chloroplast import assays from pea to maize, and we have found that hcf106 chloroplasts are defective in targeting and processing of ΔPH-dependent but not secA-dependent intermediates. One interpretation of these results is that the pH gradient is not sufficient in hcf106 mutants for ΔPH proteins to be targeted. We have measured the pH gradient in hcf106 thylakoids using radioactively labeled methylamine as a tracer molecule. Although hcf106 mutants have a reduced pH gradient when compared to wild type, the hcf106 gradient is equivalent to that in thal (cpSecA) mutant seedlings that still target ΔPH proteins normally (Voelker et al., Genetics 145: 467 [1997]). These experiments suggest that HCF106 is a component of the ΔPH targeting pathway. The hcf106-mum1 allele has been cloned by transposon tagging, and antiserum have been raised to an HCF106 fusion protein (Yonetani et al., CSHL Annual Report 1992). Chloroplast fractionation, chaotrope extraction, and protease protection experiments indicate that HCF106 is primarily a thylakoid membrane protein, with some localization to the inner envelopes. Database searches of bacterial genomes have revealed homologous open reading frames in six bacterial species, including Escherichia coli and cyanobacteria. To investigate the role of the E. coli genes, we have generated transposon insertions into this hypothetical operon. We are currently testing these strains for secretion defects.

Mutator Tagging of Genes Required for Pericarp Pigmentation

W. Flood (Dowling College), E. Grotewold

Flavonoids are a very large class of small phenolic molecules found in all plants. Flavonoids play a number of important biological functions, including plant and flower pigmentation, sexual reproduction, protection against UV radiation, defense to attack by phytopathogens, in symbiotic interactions between plants and microbes, and in the regulation of plant hormone transport. Most higher plants only accumulate 3-hydroxy flavonoids and the derived pigments, the anthocyanins (Fig. 5). Anthocyanin accumulation is controlled in maize by two classes of regulatory proteins (see Fig. 5): a Myb-domain containing class (C1 and PI) and a basic helix-loop-helix (bHLH) domain-containing class (which include the R and B genes). Anthocyanin production requires the interaction of a member of the Myb domain family and a member of the bHLH domain family.

In addition to 3-hydroxy flavonoids and anthocyanins, maize and its close relatives (e.g., sorghum) accumulate 3-deoxy flavonoids and derived pigments, which include the phlobaphenes. A single known genetic factor, P (for pericarp pigmentation), controls 3-deoxy flavonoid and phlobaphene biosynthesis. Little is known about the biosynthesis of the P-regulated phlobaphenes, which are restricted to the floral organs of maize and some other grasses. The conspicuous pigmentation determined by P in the pericarp (the pericarp is the modified ovary wall) makes it an ideal system to identify additional genetic factors required for this branch of flavonoid biosynthesis. In addition to P, factors required for pericarp pigmentation could include regulators of P expression, modulators of the P regulatory function, additional biosynthetic genes, or factors required for normal pericarp development. We started a genetic screen aimed at genetically identifying additional genes required for the accumulation of the red phlobaphene pigments in maize pericarps. We used for that purpose the Mutator transposable element system from maize. Mutator elements, different from
the Ac transposable element that we used in previous studies, have a very high rate of forward transposition with no obvious preference for linked sites. Insertions of Mutator in genes required for pericarp pigmentation should be evidenced by the characteristic and easy-to-score pericarp variegation.

We have so far screened approximately 15,000 M3+M4 ears for variegated pericarps (the pericarp is a maternal tissue, therefore the phenotype is only evidenced in the generation after the mutation occurred). We have now identified more than 30 mutations that give variegated pericarp. Allelism tests are under way to determine how many different genes have been targeted. The level and the type of variegation found in these pericarps are very different from the patterns given by Ac, as expected, given the differences in the excision properties of Mutator and Ac. Preliminary genetic and molecular data suggest that PAP1 (PAP for pericarp altered pigmentation) corresponds to an insertion (of Mutator?) in the large intron of P, providing a control that the strategy used will yield mutants in the P-regulated pathway. We are currently characterizing other PAP mutants at the molecular and genetic level.

DNA-binding Properties of the Maize Myb Domain Proteins P and C1 and Modular Structure of the Promoter of a Commonly Regulated Gene

E. Grotewold [in collaboration with M. Sainz and V. Chandler, University of Oregon]

The Myb domain proteins P and C1 independently regulate branched maize flavonoid biosynthetic pathways (Fig. 5). Whether no other genetic factor has been identified as needed for the P-regulatory function, C1 regulates transcription only in the presence of the bHLH protein R. Our previous studies have shown that P activates transcription of a1, a flavonoid biosynthetic gene also regulated by C1, by directly interacting with a1 promoter elements, which are bound with high affinity by P in vitro (hapPBS, for high-affinity P-binding sites). In addition, the DNA sequences preferentially bound by P are very different from the sequences bound by animal Myb proteins (Fig. 6) (Grotewold et al., Cell 76: 543 [1994]). Our more recent studies indicate that the cor-
responding Myb domains are solely responsible for the different in vitro DNA-binding preferences of P and v-Myb and that residues outside the conserved DNA-recognition helices are necessary for the distinct DNA-binding preferences of these two proteins (Williams and Grotewold 1997).

We have recently determined that C1 binds to the same elements as P in the al promoter, but with five to ten times lower affinity (Sainz et al. 1997). The low affinity of C1 for these binding sites was not improved in vitro by the presence of an R/B protein. C1 prefers binding to sequences very similar to those bound by P, as indicated from the results of site-selection experiments (Fig. 6). The consensus binding site for C1 indicates that C1 may have a slightly broader DNA-binding specificity than P, consistent with the larger number of flavonoid biosynthetic genes regulated by C1 (Fig. 5). The highest DNA-binding affinity of C1 is for the haPBS, indicating that the DNA-binding domains of C1 and P have a fundamental difference(s) which does not allow C1 to bind DNA with as high an affinity as P. In addition to the haPBS, the al promoter contains low-affinity P-binding sites (iaPBS), which in vivo are as important as the high-affinity sites for al activation by P (Sainz et al. 1997). Promoters containing the haPBS are also activated by C1 and R, and C1 binds the haPBS with an affinity similar to that of the haPBS. Thus, the al promoter has a modular structure with cis-elements bound by similar regulators with different affinities. We are currently investigating the mechanisms by which P and C1 regulate promoters containing solely low-affinity binding sites for these proteins.

Identification of Additional Factors Required for the Regulatory Function of the Myb Domain Proteins P and C1

M. Pizzirusso [Partner for the Future], E. Grotewold [in collaboration with Dr. Ronald Koes and Dr. Jos Mol, Vrije Universiteit Amsterdam]

The regulatory function of transcriptional regulators is often modulated by protein-protein interactions with other cellular factors and by posttranscriptional modifications. Besides the interaction of C1 with the bHLH factor R, little is known about how these processes may modulate the activity of the P and C1
Identification of Transposable Element Insertions in Maize Genes Encoding Myb Domain Proteins with Novel Cellular Functions

P.D. Rabinowicz, Y. Sun, T. Litzi (URP Program), E. Grotewold

Proteins containing the Myb DNA-binding domain, originally identified in vertebrate Myb oncoproteins, have been found in most eukaryotes, from yeast to plants and animals. Mouse and human Myb domain proteins have fundamental roles in cell cycle control and in the proliferation and differentiation of hematopoietic cells, and several lines of evidence suggest a role for Myb as a transcriptional regulator. So far, fewer than ten Myb domain proteins have been identified in vertebrates. In sharp contrast with animal cells, plants express more than 100 Myb domain proteins, and a few were shown to regulate processes such as the accumulation of secondary metabolism, the determination of cell shape and cell fate, and the control of pattern formation. The unique characteristics of these processes suggest that the increase in Myb domain protein number in plants during evolution, compared to animals, may reflect their role in the regulation of a number of cellular functions specific to plants. To investigate the function of Myb domain proteins in maize, we adapted a reverse genetics approach, originally developed in Drosophila and later used in maize, to identify insertions of Mutator transposable elements in families of genes encoding Myb domain proteins. Oligonucleotides from Mutator ends and from conserved regions in Myb domains are used to amplify by PCR pools of genomic DNA made from maize seedlings containing active Mutator elements. In 120 PCRs, 3600 plants (3600 seedlings planted in a 60 x 60 grid array) can be analyzed. By labeling the end of the Mutator-specific primer and analyzing the amplification products on sequencing-type polyacrylamide gels, we minimize the problems associated with nonspecific amplifications given by the degenerate Myb primers. Labeled bands of identical sizes present in PCRs from both dimensions of the grid constitute putative insertions in Myb-homologous sequences, allowing the immediate identification of the specific maize ear from which the kernels were planted. Using this strategy, more than ten putative insertions have been identified. From three sequenced so far, one corresponds to an insertion of a Mutator element in a putative intron of a novel myb gene. We are currently investigating whether the presence of this insertion cosegregates with a visible phenotype in the progeny of plants containing the Mutator element inserted in this gene.

Cloning of the Mixta Myb Domain Protein from Arabidopsis thaliana

P.D. Rabinowicz, G. Moriates (C.W. Post), E. Grotewold

A new area into which our research is moving is to determine the mechanisms by which Myb domain proteins regulate important plant developmental processes, in addition to the biosynthesis of secondary metabolites. Specifically, we are focusing on polarized cell growth regulated by the Myb domain proteins Mixta and GL1. The snapdragon Mixta protein regulates petal cell shape (Noda et al., Nature 369: 661 [1994]), whereas the Arabidopsis GL1 protein is required for the differentiation of epidermal cells into...
trichomes (hair cells) (Marks, Curr. Biol 4: 621 [1994]). No target genes for Mixta or GL1 have yet been identified, and little is known about the mechanisms used by plants to control cell shape, presumably by regulating the biosynthesis and/or polarized transport of cell wall components. In plants, the polarized transport of cell wall components to specific cell wall domains has fundamental developmental implications in cell shape, cell differentiation, and ultimately in pattern formation and morphogenesis. Do the GL1 and Mixta proteins regulate different aspects of the biosynthesis of specific cell wall components, similarly as P and C1 regulate different branches of flavonoid biosynthesis, or do they control trafficking of cell wall components to particular cell wall domains?

As a first step to answer this question, we cloned the Mixta gene from Arabidopsis thaliana (A. t. Mixta). A. t. Mixta contains a Myb domain 91% identical to the Myb domain from the snapdragon Mixta gene, but outside this region, the homology drops significantly (Rabinowicz et al. 1996). We are currently testing whether A. t. Mixta complements a mutation in the Mixta gene from Petunia, a plant more amenable to transformation than snapdragon.

A novel strategy to identify target genes for A. t. Mixta has been devised. We obtained from the Arabidopsis gene-trap and enhancer-trap collection at CSHL lines with GUS staining patterns specific to the petals, the tissue where Mixta is expressed. By bombarding leaves from these plants with A. t. Mixta expressed from a constitutive promoter, we are screening for lines in which GUS activity is induced by A. t. Mixta. This would indicate which plants contain an insertion of the transposable element with the reporter close to a gene downstream from A. t. Mixta.

Experiments are also currently under way to determine the effect of the ectopic expression of A. t. Mixta in leaf epidermal cells and how mutations that affect trichome formation interfere with Mixta function.

**PUBLICATIONS**


In Press


**ARABIDOPSIS FLOWER DEVELOPMENT**

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We continue to probe the molecular mechanisms controlling cell fates during development, using Arabidopsis thaliana. Specifically, we focus on genes regulating flower development. During the past
several years, we have been characterizing the function of several members of the MADS-box gene family, which has members in all major eukaryotic kingdoms. One of the genes that we study is the Arabidopsis floral homeotic gene AGAMOUS (AG), which is a critical regulator of both floral organ identity and floral meristem identity. Many other MADS-box genes were isolated molecularly and designated as AGL for AG-Like.

In addition, we have isolated and characterized three Arabidopsis mutant alleles in the FON1 gene (Floral Organ Number); these mutants have an increased number of reproductive floral organs. Furthermore, we are in the process of identifying new genes required for flower development using enhancer trap/gene trap transposon mutagenesis methods. In addition, to isolate new genes that function during late floral organ development, we have identified a number of genes specifically expressed in carpels or stamens. These genes are likely to be involved in cell differentiation during late flower development, and they provide molecular markers for specific cells or tissues of the reproductive organs.

In 1996, we were joined by two new postdocs, Ming Yang and Dehua Liu, in April and June, respectively. Ming had just finished his Ph.D. at Ohio State, where he studied Arabidopsis mutants defective in cell division patterns in leaves, and Dehua was a postdoc at the Noble Foundation in Oklahoma, where he studied an enzyme involved in fatty acid biosynthesis. We bid farewell to our postdocs Cathy Flanagan, who moved to the Seattle area with her husband Andrew Flint, and Yukiko Mizukami, who went to Berkeley to continue her research in the area of plant reproductive development. Last year, as in previous years, several undergraduate students participated in our research. From SUNY at Stony Brook, Kristen Grace and Julissa Cruz came to our lab in the spring and continued in the summer, Harpreet Katari and Robert Levin joined us in the summer, and Julissa Cruz, James Lyons, Danielle DeLagante, and Samit Guha worked here in the fall; they participated in our enhancer trap projects and transgenic plant work with the AG and AGL genes. Aliyah Hameed from Bryn Mawr returned for a second summer, working with postdoc Hai Huang. David Kwan from Berkeley also spent part of the summer working with Hua-Ying Fan, a postdoc in our lab, to characterize the interaction between AG and AGLs. Last spring, our Partner for the Future, Dominik Rosa, a senior at Commack High School, completed his work with Peter Rubinelli on stamen-specific cDNA clones. And last fall, a new Partner, Sophia Virani from Herricks High School, started working on the analysis of stamen-specific genes and the AGL1 gene. We gratefully acknowledge the assistance of Upland Farms’ manager Tim Mulligan and his assistant Stan Schwarz.

**Generation of New Enhancer and Gene Trap Transposants and Analysis of Floral Expression Patterns**


To identify new genes regulating Arabidopsis flower development, we have continued to generate enhancer trap/gene trap transposon insertion lines. We have now generated about 2200 independent insertion lines, or transposants. At the same time, we have begun to characterize the transposants. We have examined the expression patterns of the Ds-borne GUS reporter gene in the first 900 transposants generated by the laboratories of Drs. Sundaresan, Martienssen, and Grossniklaus, and our lab. We have examined the staining patterns in the inflorescences, individual mature flowers, seed pods, and cauline leaves (as a control for vegetative tissue). Overall, about 20–30% of the transposant lines stain in the flower. GUS staining was found in flowers at early, intermediate, or late stages of development, and various combinations of staining in different organs were observed. In particular, patterns in the reproductive organs and at intermediate to late stages are the most common. On the other hand, truly constitutive expression was rare. Furthermore, there are many different patterns within single organs, reflecting cell- or tissue-specific GUS expression. The characterization of these and additional lines should allow us to identify many new genes functioning in flower development.

**AG-AGL Interactions Mediated by the K Domain**

H.-Y. Fan, Y. Hu, M. Tudor, D. Kwan, H. Ma

Plant MADS domain regulatory proteins have a region of moderate sequence similarity that has been
designated as the K domain, and its predicted coiled-coil structure suggests a role in establishing a protein-protein interaction. In vivo studies with the Arabidopsis AGAMOUS (AG) protein have indicated that the K domain is important for AG function. Using a bait fusion protein containing the K domain and the carboxy-terminal region of AG in a yeast two-hybrid selection, we found 156 clones that encode potential AG-interacting proteins. These clones encode one of four highly related MADS domain proteins: AGL2, AGL4, AGL6, and AGL9. Additional analysis showed that the K domain of AG alone was able to bind the K domains of these AGLs. This binding was further confirmed by immunoprecipitation experiments using in-vitro-synthesized AG and AGL proteins. These results strongly indicate that AG interacts with AGL2, AGL4, AGL6, and AGL9. On the basis of these results and previous observations, we propose that AG function requires interaction with at least one of these AGL proteins, and such interactions contribute to the functional specificity of the AG protein.

Phenotypic Analysis of Double Mutants between fon1 and Other Floral Mutations
H. Huang, A. Hameed, H. Ma

We previously reported the isolation of an Arabidopsis floral organ number mutant, fon1, and the characterization of its phenotype (H. Huang and H. Ma, 1993, 1994, 1995 Annual Reports). The fon1 single-mutant phenotype indicates that the FON1 gene regulates the duration of the floral meristem, and the organization of floral organ primordia. To test for genetic interaction between FON1 and other genes, we have generated and analyzed a large number of double mutants. The clavata (clv) mutations are known to affect floral meristem activity. We found that the clv1 fon1, clv2 fon1, and clv3 fon1 double mutants all show similar phenotypes, having more stamens and carpels than either fon1 or clv single mutants. These results indicate that FON1 and CLV genes function in different pathways to control the number of third and fourth whorl floral organs. In addition, double-mutant phenotypes suggest that FON1 does not interact with TERMINAL FLOWER1 (TFL1), APETALA1 (API), APETALA2 (API2), or UNUSUAL FLORAL ORGAN (UFO). In contrast, normal LEAFY (LFY) function is required for the expression of fon1 phenotypes. In addition, FON1 and AGAMOUS (AG) both seem to affect the domain of APETALA3 (AP3) function, which also affects the formation of stamen-carpel chimera due to fon1 mutations. Finally, genetic analysis suggests that FON1 interacts with SUPERMAN (SUP), which also regulates floral meristem activity.

Characterization of Organ-specific cDNAs
P. Rubinelli, Y. Hu, S. Virani, D. Rosa, H. Ma

As described in previous Annual Reports, we have been characterizing several cDNAs that are expressed specifically or predominantly in anther or pollen. Among these clones, APT27 encodes a putative glucosidase with similarity to plant glucosidases that convert inactive storage forms of the plant hormone cytokinin to active forms. We have constructed Escherichia coli expression plasmids to produce recombinant APT27 protein for in vitro biochemical studies, which are in progress. At the same time, several constructs have been made to test its function in vivo using transgenic plants. We are currently generating transgenic plants. In addition, antisense transgenic plants are being produced to test the function of a few other anther/pollen-specific genes.

Characterization of Male Fertility Mutants
M. Yang, H. Ma

To identify and characterize new genes that control the development of the male reproductive organ stamen and male haploid generation (gametophyte) pollen, we have searched for male sterile or low-fertility mutants among the enhancer/gene trap transposants. More than 20 candidates were found thus far, and we have characterized two of these in some detail. The first mutant, 218-181, has a reduced fertility as indicated by a reduced seed set. The mutant also has defects in floral organs, including fused sepals and filaments. We found that the filament of the mutant stamens has about one-fourth the normal length and that pollen development per se
seems to be normal and the pollen grains are functional. The fertility defect seems to be due to an inability to deliver pollen grains to the stigma. The second mutant, 1176-306, is a tight male sterile mutant. The mutant also produces smaller petals. Microscopic analysis indicates that pollen development and/or release is affected. The mutant filament is about half as long as the normal one and has about half as many normal-sized cells as in wild type. Therefore, cell division may be affected in the mutant filament and possibly in the mutant petal. The sterility could be due to the same cellular defects during pollen development.

**PUBLICATIONS**


In Press


**HIGHER-ORDER CHROMOSOME DYNAMICS**

T. Hirano M. Hirano
K. Kimura
A. Losada

Our laboratory is interested in understanding the molecular mechanisms responsible for the dynamic changes of higher-order chromosome structure during the cell cycle. The experimental system we use is a cell-free extract derived from *Xenopus laevis* (African toad) eggs in which higher-order chromosome structures can be reconstituted in vitro in a cell-cycle-dependent manner. This unique system provides us with both a simple procedure for chromosome isolation and a powerful functional assay for chromosome condensation. We previously identified major polypeptides of mitotic chromosomes assembled in this cell-free system, which we referred to as *Xenopus* chromosome-associated polypeptides (XCAPs). Our initial efforts were focused on two of the XCAPs, XCAP-C and XCAP-E, and we were able to demonstrate that they have a key role in mitotic chromosome condensation in vitro. Both polypeptides belong to an emerging family of putative ATPases, the SMC (structural maintenance of chromosomes) family. The family has expanded rapidly in the last 2 years since its discovery, and it is now known that SMC proteins are involved in many aspects of higher-order chromosome structure and function, including chromosome segregation, dosage compensation, and recombinational DNA repair. During the past year, we purified protein complexes (termed condensins) containing XCAP-C and XCAP-E and found that three additional components copurified with these polypeptides. We completed cDNA cloning of the newly identified subunits and obtained basic information on DNA-binding activities associated with condensins. We also extended our efforts to the identification and characterization of a second class of SMC protein complexes in *Xenopus* egg extracts.

Molecular Characterization of Condensin Subunits

T. Hirano, M. Hirano [in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory]

By immunoaffinity chromatography using anti-peptide antibodies, we purified protein complexes containing XCAP-C and XCAP-E from *Xenopus* egg extracts. Sucrose density gradient centrifugation of an
affinity-purified fraction revealed two major forms of the complexes: The 8S form (termed 8S condensin) is a heterodimer of XCAP-C and XCAP-E, whereas the 13S form (13S condensin) contains three additional subunits. We found that the newly identified subunits are identical to previously uncharacterized XCAPs, XCAP-D2, XCAP-G, and XCAP-H. Both 8S and 13S condensins are targeted to chromosomes in a mitosis-specific and phosphorylation-dependent manner. Immunodepletion and addback experiments showed that 13S condensin is absolutely required for chromosome assembly in vitro. Our results also suggested that 8S condensin has a minor contribution to proper condensation of mitotic chromosomes.

We cloned cDNAs encoding the newly identified three XCAPs and found that all of them are novel proteins (Table 1). An initial database search identified homologs of yeast and humans, but no genetics or biochemistry had been described in these homologs. Very recently, however, an apparent homolog of XCAP-H was identified from Drosophila melanogaster as a gene product, Barren, whose mutation shows a defect in mitotic chromosome segregation in early embryos. Although biochemical characterization of Barren remains to be done, the mutant phenotype and the chromosomal localization of Barren are totally consistent with the idea that it is a component of 13S condensin in Drosophila. We therefore anticipate that the structural organization and individual subunits of condensins are highly conserved among eukaryotes and that they play a fundamental part in chromosome condensation both in vitro and in vivo. Moreover, we found a limited similarity between XCAP-H and DPY-26, a Caenorhabditis elegans protein required for meiotic chromosome segregation. Remarkably, in somatic cells, DPY-26 is also involved in dosage compensation as a component of a protein complex containing DPY-27, an SMC protein exclusively required for dosage compensation. This result strengthens our previous proposal that the two apparently different chromosomal events, chromosome condensation and dosage compensation, might share common molecular mechanisms.

### Biochemical Activities of Condensins

K. Kimura, T. Hirano

Although we have demonstrated that condensins are able to drive chromosome condensation in our cell-free system, we do not know how they work in this complex process. To understand condensin functions at a mechanistic level, we are trying to identify biochemical activities of purified condensins. We have made three predictions for functions of this novel class of protein complexes: (1) Since genes encoding SMC proteins have been found in several bacterial genomes, the primary target of condensins is DNA, rather than eukaryote-specific chromatin components (e.g., histones); (2) since condensins localize to whole chromosomal arms, they are not likely to exhibit strict sequence specificity for their DNA-binding; and (3) since XCAP-C and XCAP-E contain putative ATP-binding motifs, condensins must have an activity that changes DNA structure in an ATP-dependent manner. We tested these predictions by using affinity-purified fractions containing 13S condensin. We found that the fractions contained a DNA-binding activity as determined by a gel retardation assay using nonspecific duplex DNA probes. Competition experiments showed preferential binding to long DNA fragments compared with short ones (e.g., 600 bp vs. 100 bp). We also found that the 13S condensin fractions exhibited higher affinity for synthetic cruciform DNAs than for duplex DNAs with the same sequences. Thus, 13S condensin might recognize structured DNAs, such as DNA crossover or bent DNA. Furthermore, preliminary experiments suggested that 13S condensin was able to introduce positive supercoils into a closed circular DNA in the presence of topoisomerase I. The supercoiling reac-

### Table 1 Subunits of the Two Different Classes of SMC Protein Complexes

<table>
<thead>
<tr>
<th>Molecular Mass</th>
<th>S. cerevisiae</th>
<th>S. pombe</th>
<th>D. melanogaster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenopus mass</td>
<td>S. cerevisiae</td>
<td>S. pombe</td>
<td>D. melanogaster</td>
</tr>
<tr>
<td>XCAP-C</td>
<td>165 kD</td>
<td>Smc4pB</td>
<td>Cut3p</td>
</tr>
<tr>
<td>XCAP-E</td>
<td>140 kD</td>
<td>Smc2p</td>
<td>Cut14p</td>
</tr>
<tr>
<td>XCAP-D2</td>
<td>150 kD</td>
<td>yes</td>
<td>?</td>
</tr>
<tr>
<td>XCAP-G</td>
<td>130 kD</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>XCAP-H</td>
<td>100 kD</td>
<td>yes</td>
<td>?</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Subunits of the XSMC1/3 complexes</th>
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<tbody>
<tr>
<td>XSMC1</td>
</tr>
<tr>
<td>XSMC3</td>
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*Yes indicates that a putative homolog of unknown function has been deposited in the databases.*

* Determined by SDS-PAGE.

* Named, but no functional analysis has been reported.*
tion was ATP-dependent. We speculate that 13S condensin either wraps or overwinds DNA by utilizing the energy of ATP hydrolysis, thereby inducing local positive supercoiling of chromosomal DNA. Such topological changes could lead to an accumulation of torsional stress of DNA in adjacent regions, which in turn contributes to higher-order structural changes of chromatin fibers. Experiments to test this model are now in progress.

Identification of a Second Class of SMC Protein Complexes

A. Losada, M. Hirano, T. Hirano

The genome project of Saccharomyces cerevisiae identified four members of the SMC family in this organism, Smc1p, Smc2p, Smc3p and Smc4p. Previous genetic studies showed that Smc1p and Smc2p are required for proper segregation of mitotic chromosomes, whereas mutations in Smc3p and Smc4p have not yet been reported. Since Smc2p and Smc4p are most homologous to XCAP-E and XCAP-C, respectively, we believe that the two yeast gene products function as core subunits of yeast condensins (Table 1). This is consistent with the previous observation that a defect in chromosome condensation was observed in smc2, but not in smc1. What then is the exact role of Smc1p and Smc3p in chromosome dynamics during mitosis? This question prompted us to search for additional SMC polypeptides from Xenopus egg extracts. On the basis of sequence homology, we cloned cDNAs encoding XSMC1 and XSMC3, Xenopus homologs of Smc1p and Smc3p, respectively (Table 1). By raising specific antibodies against these polypeptides, we found that XSMC1 and XSMC3 associate with each other in the extracts, forming two distinct complexes with sedimentation coefficients of 10S and 15S. Neither the 10S nor 15S form contains condensin subunits. Conversely, neither XSMC1 nor XSMC3 associates with condensins. By analogy to 13S condensin consisting of five subunits, it is likely that the 15S complex contains additional subunits besides XSMC1 and XSMC3. We are now investigating the localization and functions of these new protein complexes containing XSMC1 and XSMC3. Preliminary results suggested that the XSMC1/3 complexes are not required for chromosome condensation in our cell-free system. We expect that they might be involved in other processes essential for proper segregation of mitotic chromosomes, for example, sister chromatid cohesion.

PUBLICATIONS

STRUCTURE AND COMPUTATION

Scientists in this section are engaged in basic research and methodology development in gene discovery and in gene and protein functional analysis. The program is comprehensive and has major programs both in the experimental and computational aspects of gene discovery and in the analysis and prediction of gene and protein structure and function. The main scientific thrust and overall goals of this section are to develop and apply methods that

- establish chromosomal position of heritable disease genes by linkage and segregation analysis of affected families
- automate high-speed DNA sequencing over long contiguous regions of genomic DNA
- automate the detection of segments of genomic DNA sequences that have specific biologic content
- predict gene function by homology/analogy to existing sequences
- isolate and characterize minuscule amounts of proteins for cloning genes using degenerate PCR techniques
- use X-ray diffraction patterns with crystalline proteins to determine their three-dimensional structure with the goal of understanding their function
- use combinatorial chemistry methods to screen for significant molecular interactions among large arrays of molecules

COMPUTATION AND HUMAN GENETICS

T. Marr  S. Cozza  J. Helmich  J. Salit
D. Cuddihy  T. Iwasaki  A. Tracy
D. Gordon  J. Lugert  M. Zhang
E. Hackborn  M. Mallison

GENOME TOPOGRAPHER SOFTWARE

The overall goal of our laboratory is to enhance the capability of the genome topographer (GT) prototype software for use as a high-quality software package that addresses major informatics research requirements of the molecular biology and genetics research and development communities. GT is a major software package that we have been working on for several years. We have major funding through the National Institutes of Health (NIH) as a Human Genome Informatics Research Resource and from Genomica Corporation. This funding relationship between NIH and the private sector was strongly encouraged by the NIH Council. This software is designed to be useful to users ranging from those in large-scale production labs to those in smaller, hypothesis-driven labs.

In recent years, many of the molecular details of the structure of the human genome have become apparent as a surge of research has produced new information about chromosome organization and genetic and physical maps, and details about specific chromosomal regions, gene sequences, and gene/protein interactions. These types of information are being used to drive research in several areas, much of it medically oriented. Specifically, research into the genetic basis of complex diseases is heavily influenced by these recent advances.

A disease is considered complex if it displays non-Mendelian characteristics. In general, this is the result of a disease phenotype being caused by the interaction of multiple genetic and environmental factors. More specifically, this may happen because different mutations at a single locus give rise to clinically similar phenotypes (allelic heterogeneity); mutations at any one of a number of different loci cause clinically indistinguishable phenotypes (locus heterogeneity); differences in expression of an allele depend on
which parent transmitted it (genetic imprinting) or expression of the gene is affected by the sex of the individual (sex limitation); expression of a mutant gene causes disease in only some individuals (incomplete penetrance); inheritance is strictly maternal (mitochondrial inheritance); expression of an allele depends on interactions with other genes and environmental factors (multifactorial inheritance); expansion of trinucleotide repeats in coding or regulatory regions of the gene alters its expression in a progressive manner over several generations (anticipation); the occurrence of environmentally induced nonheritable cases of a disease with a phenotype indistinguishable from the genetic version (phenocopies); or novel mutations in a germ line or tissue cause disease (sporadic cases). Complex disorders may also be plagued by problems of uncertain diagnoses because of the range of phenotypic variation and variable age of onset.

In simplistic terms, it may be considered that there are two types of complex diseases. The first would be those that show a relatively high level of heritability and that may be studied primarily by tracking a disease gene through generations of families that display familial clustering. The second would be those that are far more frequently the result of the accumulation and interactions of somatic mutations. Here, molecular and biochemical analyses represent a feasible approach to study.

A group of diseases that fall into the first category is the human psychiatric diseases. It has been demonstrated through twin and family studies that many of these diseases have a considerable genetic component, which makes them reasonable candidates for the genetic linkage. Many exhibit a bewildering array of phenotypes, variable age of onset, heterogeneity, polygenic inheritance, and incomplete penetrance. These factors combined have made analysis of genetic data difficult using the available technology and have drastically slowed progress in disease gene localization. Many of these disorders are very prevalent in the population. For example, bipolar disorder has an estimated prevalence of 1–2% in the general population. Therefore, these diseases represent difficulties and medical expenses for a large segment of the general population.

The other type of disease is typified by cancer, a clearly complex disease characterized by uncontrolled or otherwise abnormal proliferation of cells. The progression of the disease involves the escape of a single cell from its normal proliferative controls, clonal amplification of the altered cell and accumulation of secondary mutations, bypass of the organism’s defenses that otherwise eliminate abnormal cells, metastasis of the primary tumor to other parts of the body, proliferation of the metastatic cells in other organs and elimination of function in these organs, and the development of multidrug resistance. Current estimates of the number of genetic alterations that are required for this full progression range from three to five.

These disorders typify two poles of medical research today and are used to guide the development of GT as a useful computer tool. The new areas we emphasized most strongly this past year are the generation of a linkage analysis workbench, the generation of a sequence analysis workbench, and the development of specialized database tools that integrate information that might lead to novel breakthroughs or novel experimental ideas in the study of collections of genes and their products such as oncogenes. These represent three facets of research where the application of sophisticated computer technology will contribute significantly. Although they are being developed separately, it must be stressed that the combination of such approaches and tools is the real strength of GT, and the study of any disease should be facilitated by the use of one or more of them.

The development of the linkage analysis workbench will address the current technical difficulties encountered in carrying out linkage, epidemiology, and segregation analysis. The details of the design of this package can be seen in the user manual overview pages, but it has become increasingly clear that a real need exists for the standardization, integration, and simplification of computer-based approaches to these analyses.

The study of diseases such as cancer is not as well suited to linkage analysis techniques and would benefit more from the integration and correlation of data relating to cellular biology (for information on the genetic, molecular, and biochemical basis of cellular proliferation and invasion), immunology (for information on the body’s defense mechanisms for elimination of abnormal cells), and epidemiology (for information on environmental risk factors and genetic predispositions present in subpopulations).

The role of rapid and complete sequence analysis in both genetic and sporadic lesion-based disease research cannot be overstated. Sequence analysis becomes critical at the point where disease genes have been identified and at the point where gene/gene interactions are being assessed. Apart from annotating the sequence of a new gene, sequence analysis
permits researchers to define functional families of proteins that in turn provides some additional information about the biochemical interactions that underlie the disease. From the results of database searches, one may locate sequence motifs, make functional predictions, and functionally delineate protein families. Rapid iterative testing of slight variations of a motif to improve its specificity would be one way to exploit sequence analysis methodologies to augment the amount of information gained.

There is now a great need for the type of integrative and analytical software that we are proposing to develop. Currently, researchers must use several different sources of data to devise informative experiments, such as the published scientific literature and scientific meetings. They must be familiar with a myriad of often difficult-to-use computer programs to perform even relatively simple investigations into the potentially relevant record of data or to perform analyses on data that they have collected. Limitations also apply to the interpretation of results, which would be simplified and enhanced by allowing researchers to see them in the context of what is currently known about the genomes of human and model organisms. The consequence of the current situation is that most biologists are not able to efficiently and fully analyze their data and are likely missing crucial new findings. The situation is exacerbated by an ever-growing number of journals, scientific meetings, specialized databases using ad hoc nomenclature, data representational and computer access methods, and analysis programs that have individual idiosyncratic requirements.

Considering these facts, a major goal in the development of the GT software has been to allow the user to move from clinical assessment of disease phenotypes, to genetic map, to physical map, to sequence and function, and back to clinical assessment of potential drugs using what appears to the user as one computer program. Our work in 1996 extends this idea to support the movement from sequence or linkage analysis programs to stored genome data or the movement from specialized databases to the generally available public data servers with the same ease. Of critical importance is the fact that GT will allow researchers to maximally utilize the conservation of genes and the chromosomal synteny of model organisms, such as yeast and mouse, quickly and easily. The additional data that are available from such model organisms are currently not fully exploited by many researchers and will likely provide critical insights into existing data.

The next phase of the Human Genome Project (HGP) is likely to be characterized by the availability of (1) significant amounts of DNA sequence from human and model organisms, (2) physical maps of increasing resolution with localized ESTs of the human and mouse genomes using standardized, verifiable mapping technologies, (3) high-density maps of polymorphic genetic markers for the human and mouse genomes, and (4) major advances in methods for high throughput genotyping, positional cloning and/or exon trapping, and sequencing.

The methods and materials resulting from the HGP are well suited to meet the challenges in the study of complex human genetic diseases, which require the ability to detect and sort out heterogeneous, polygenic, and epistatic genetic effects, modulated by environmental factors. Scientific investigations into the parts of the puzzles presented by these complex problems require application of state-of-the-art experimental methodologies in the fields of genetics, molecular biology, and biochemistry. It is our thesis that gene finding, functional analysis, and efficacy testing could be greatly accelerated using state-of-the-art computer methodologies if (1) relevant data were represented in a simple, reproducible, and semantically integrated manner, (2) the timeliness of the flow of such data between small, hypothesis-driven research labs and large genome centers was optimized, and (3) basic researchers were empowered with "user-friendly," but technically powerful, tools for computer-aided interpretation of the data. These are the goals of our GT software project.

The software allows users to take full advantage of the data being generated by International Human Genome Programs. The scope of the software includes applications in clinical genetics and epidemiology, statistical genetics, molecular biology, and molecular genetics and biochemistry. The software has been in field test at several locations since October 1996, and the first production release 1.0 of this client-server based software, which is scheduled for release in third quarter 1997, will allow users to carry out the following:

1. Connect by internet/intranet to a central GT database server for access to semantically integrated collections of most of the large-scale data currently generated by the international genome project. Example data sources include the Whitehead/MIT Center, GDB, Genethon, Sanger Center, Jackson Lab Mouse Genome Database, and all of the nucleotide and protein sequence databases at NCBI.
2. Graphically display and execute queries for selectively sifting through these large and sometimes redundant data collections, retrieving results into the GT client application, cut-and-paste results into other client applications, such as word processors, and print postscript graphics of a comprehensive collection of genetic, radiation hybrid, and physical maps, including highly annotated genomic sequences, for example.

3. Query and integrate results from the NCBI MEDLINE and sequence databases using keywords and/or by searching them using BLAST. In addition, for users with adequately equipped Unix workstations, we provide the option to perform rigorous sequence database searching and alignment studies using FASTA and a full dynamic programming method developed at the Resource. This latter option is particularly useful for STS and EST analysis.

4. Transparently connect to multiple GT servers so that users can go to original sources of data (which use a GT server). Ultimately, GT will run in a truly federated mode whereby the user is capable of transparently running queries across multiple geographically distributed databases of mixed architecture.

5. Use GT experiment management capabilities for managing, tracking, and assessing the quality of large-scale DNA mapping and sequencing projects.

The first official test release of the GT software was October 1996. It was installed and tested at six laboratories representative of the wide range of users intended for GT. Following the October 96 release and after getting feedback from user sites and making appropriate changes to the software, we released and began installation of version 1.0 gamma of the GT software in March 1997 to an expanded 15 test sites, including three pharmaceutical companies, two biotech companies, two private medical research laboratories, one government medical research laboratory, and seven intermediate sized, academic molecular biology and genetics research laboratories. We refer to these 15 sites as "opinion leader" sites because they all are major sites and involve opinion-leading scientists. A full-featured user manual, administrators’ manual, and training guide have been written.

Perhaps the most exciting and largest technical advance in the underlying GT technology was the arrival of our vendor-based technologies: (1) database federation technology available in the GemStone database product, and thus in the GT server, and (2) the Common Object Request Broker Architecture (CORBA). The short- and long-term implications of these technologies are wide ranging and include the potential development of a Federation of GT database servers and clients that are all transparently interconnected semantically, logically, and securely. This could lead to the consolidation of the informatics needs for data generation and distribution of as much as is desired by the genome program management, and perhaps as important, related programs taking advantage of genome program data.

For example, using a GT Federation system, it would be possible to put a GT server in several human genome sequencing centers which will transform clones from a sequence-ready physical map into long sequences of genomic DNA. People at the NCHGR Sequencing Center would probably be involved in the further analysis and annotation of sequences by computer and some experiments. It would be useful to have a great deal of peer-reviewed scientists at other sites analyzing these sequences as well, because of the difficulty in automated feature extraction from mammalian sequences. Federated GT servers and clients could be transparently integrated to many such sites. Furthermore, suppose that a peer-reviewed scientist (e.g., NCI study section) is studying sequence polymorphisms in large collections of oncology patients and their tumors pathologies. A GT server and client could be provided and transparently integrated into the growing Federation as well. None of the growing or incrementally increasing knowledge base need always be available for perusal by others on the Federation network, or the public in general using GT security tools, but rather only after the data and metadata have undergone some agreed upon release mechanism. This is only one scenario using this powerful new database Federation technology. The new CORBA interface opens up other, but equally powerful, possibilities.

We have been working with two different new releases of CORBA, one at the server level and one at the client level. So far, we have concluded that the server-level implementation is the most sound given the state of CORBA technologies as a whole. The CORBA implementation that GemStone supplies with their release 5.1 product provides an industry standard ORB (such as standards are) and supports language interfaces including Smalltalk, Java, C, C++, and HTML. For our own development process, this allows us to use CORBA for all network-based
computing, such as our compute server applications, using a very sophisticated application program interface that includes queuing, security, failure, and most asynchronous network processing using industry-standards. We have developed some of these ourselves in the past. More importantly is that CORBA opens up GT to outside developers so that they can write their own applications against GT without interaction with the GT development team. It also means that both HTML and Java based browsers can be written using the GT databases for the data server, without using the GT client at all.

The Genetic Basis of Manic-Depressive Illness

A Charles A. Dana Foundation Consortium among Cold Spring Harbor Laboratory, Johns Hopkins University, and Stanford University.


(1) Cold Spring Harbor Laboratory, Structure and Computation Program
(2) Johns Hopkins University, School of Medicine, Department of Psychiatry
(3) Stanford University, School of Medicine, Department of Human Genetics

The Dana Consortium is formally coming to an end in June 1997, but data analysis will be an ongoing effort at all of the contributing labs. Positive results have now been repeatedly found on chromosome 18q. This may be the first such finding ever for a gene involved in a complex human behavior.

Because of the expected complexity of the genetic components of manic-depressive disorder (MDD), it is essential that the clinical and experimental data that provide the foundation of the genetic study be of highest quality possible. Furthermore, because of the lack of mathematical algorithms that are clearly superior, or perhaps even relevant, to detecting linkage between phenotype and genotype for genetically heterogeneous disorders, such as MDD is thought to be, it is essential that the basic data be managed in a computer system that allows for facile manipulation and extensive, flexible analysis of the data. Finally, again because of the lack of rigorous analytic tools, it is essential to have a computer system that allows for extensive manual, visually based exploratory analyses.

These are the issues that Cold Spring Harbor Laboratory has focused on in this collaboration, namely, providing a rigorous data handling, manipulation, and analysis computer platform for the ongoing and final phases of the project. We have developed a software system that addresses these issues, which will be critical to the next phase of the project, i.e., the analysis phase. We will distribute our software to the collaborators after most of the basic data have been collected and entered into the system, and analyses can then proceed. As discussed with Dana officials at the beginning of this project, the system we developed is generally applicable to complex genetic systems and is not specific to MDD and will therefore be useful to potentially a large number of important genetic disorders, including other neurologic disorders, cancer, and hypertension.

The types of data that needed to be supported in our project are extensive and include clinical data, pedigree data, genotyping data, laboratory data concerning locations and identities of cell lines and DNA supplies, and related matters. We have developed an integrated computer program that now is capable of storing, manipulating, consistency checking, and otherwise generally analyzing all of the relevant primary data resulting from the clinical and experimental efforts of the Dana Consortium. The software is distributed on a CD ROM disk and can be easily installed by relatively novice computer users. The client software runs equally well on Macintosh, PC, and Unix computers. The database software runs on PC computers running MS Windows NT or Unix systems.

Because there are no existing mathematical methods that are clearly superior to others or are known to be singly effective in linkage studies in diseases that are genetically heterogeneous, we have developed new methods that determine linkage and/or enhance current linkage methods. We have used mathematics and statistics to provide analytic solutions to questions about distribution theory, power, and sample size for linkage tests. We are currently applying these methods to the Dana bipolar data under a range of genetic models and parameter specifications.

For the data analysis portion, we have performed the following: an exploratory data analysis of certain genetic model-based and genetic model-free statistics from current state-of-the-art linkage programs SAGE SIBPAL. (Tran et al., Sib-pair Linkage Program Version 2.6. Part of S.A.G.E. Release Documentation 1384 [1994]), and GENEHUNTER (Kruglyak et al., Am. J. Hum. Genet. 58: 1347 [1996]). We have applied analysis of variance with markers and pedigree effects, Tukey’s one-degree-of-freedom test for removable additivity, and transformation of the data.
to remove nonadditivity. We have run SAGE SIBPAL on the entire genome. Our findings agreed with the results published by Stine et al. (Am. J. Hum. Genet. 57: 1384 [1995]), namely, strongest evidence for linkage was on chromosome 18, the pter (marker D18S37). It is vitally important that data analysis of the Dana families be continued. The findings of Stine et al. were marginally significant, and our exploratory data analysis in combination with our mathematical computations will enable us to determine if the finding is a true but weak finding or a false-positive finding. Furthermore, our methods will enable us to determine if other chromosomes suggest linkage to bipolar illness.

We have studied the effects of allele frequency variation on the outcome from linkage tests. A common practice among researchers performing linkage studies is the use of equal allele frequencies as input when reporting P-values from computer linkage programs. Ott (Am. J. Hum. Genet. 51: 283 [1992]) demonstrated that in the presence of untyped individuals, falsely assuming equal allele frequencies tends to lead to strong false-positive evidence for linkage in parametric lod score analysis. To address the same question in nonparametric tests, we (Gordon et al. 1997a) performed an analysis using a uniform prior distribution of allele frequencies on the test results from the linkage program SAGE SIBPAL, which tests for proportion of alleles identical-by-descent among affected siblings, and which also performs the Haseman-Elston test. Our data were taken from a NIMH bipolar study (Berrettini et al., Proc. Natl. Acad. Sci. 91: 5918 [1994]), specifically family genotype data for the marker ACTH112. As part of our study design, we removed genotype information from randomly selected parents in these pedigrees and observed the effects that such missing data would have on the test results. Our results showed that the P-values resulting from equal allele frequencies as input were never the most conservative. In fact, for the Haseman-Elston test, at least 50% of the P-values were larger than the equal-allele-frequencies P-values (see Table IV in Gordon et al. 1997a). In addition, we found that the SAGE SIBPAL test for proportion of alleles identical-by-descent among affected siblings showed a greater percentage of suggested linkage with decreasing parental genotype information, whereas the Haseman-Elston test results produced suggested linkage comparatively less frequently.

We will extend this work by performing a similar analysis on simulated data in which the genetic model is known, the true allele frequencies are known, and complete genotype information exists on all of the parents in the study.

Using mathematics, we have computed the first four moments of a quadratic form when its input variables are independent (Gordon et al. 1997b). Given this hypothesis, we can approximate the null distribution of any quadratic form of these response variables and calculate significance levels, perform power calculations, and develop a deeper understanding of the properties of nonparametric linkage tests such as the Haseman-Elston test (Haseman and Elston, Behav. Genet. 1: 3 [1972]). Continuation of these studies is essential for the eventual success of the project.

In Press


SEQUENCE-BASED ANALYSIS OF COMPLEX GENOMES

W.R. McCombie  N. Dedhia  A. Johnson
S. Granat  N. Kaplan
J. Hoffman

The long-term goal of our lab is to utilize the latest technology of automated DNA sequencing to determine the correlation between structure and function in complex genomes. This involves generating genome sequence data as well as the development of new tools to generate and analyze these data. This summary is accordingly divided into development and sequencing sections.
TECHNOLOGY DEVELOPMENT AND IMPLEMENTATION

Software Development

N. Dedhia, W.R. McCombie

An important facet of successful large-scale sequencing is the ability to sequence a large number of samples consistently. Tracking data flow in high-throughput sequencing is important in maintaining a consistent number of successfully sequenced samples, making decisions on scheduling the flow of sequencing steps, trouble-shooting problems at various steps, and tracking the status of different projects. We had already developed an informatics structure capable of dealing with our previous data flow, but our anticipated scale-up would require a more sophisticated system. In addition, we wanted to use the latest sequence assembly software and associated tools to facilitate this important process. These tools also required a different computational environment from the one that we had. As a result, we decided to perform a major reconstruction of our informatics infrastructure. We obtained several relevant Unix scripts from our collaborators at the Genome Sequencing Center at Washington University. They developed these scripts to move and reformat sequencing data. Most of these scripts contained code specific to the setup at Washington University. We modified the code to fit the data flow system we were designing. We used these scripts, the latest sequence assembly software (PHRED and PHRAP from Phil Green), and portions of our original data flow software, to construct our new system. This system was originally constructed so that the data processing operations would be initiated by a Unix-based command line interface. This portion of the project has been completed and the next step is to implement a Netscape-based system that will place the entire process of data flow monitoring and control under a Web-based interface. This will simplify the user interface significantly as well as link to our sequencing group Intranet that is under development. The new Web-based software package we are developing is modular and designed to allow additional unit operations to be added and tracked with ease. The software can be interfaced with different base-calling and assembly engines. Site-specific details are sequestered and can be customized in a simple manner. Access to the underlying relational database is currently through the Perl DBI interface, thereby allowing the use of different relational databases.

Development of a High-throughput Sequencing Infrastructure

A. Johnson, N. Kaplan, S. Granat, J. Hoffman, W.R. McCombie

A major part of our effort in 1996 was devoted to scaling up our sequencing efforts. In the summer of 1996, we moved into a new lab in the Hershey building and roughly tripled our size. This required different project management methods than those used for smaller projects. Much of this was in terms of software development as described. However, substantial modifications to almost all of our laboratory procedures were necessary to allow us to scale up the sequencing and reduce the cost per base at the same time.

Our template purification methods were completely changed both to allow higher throughput per per-
son and to reduce cost. Previously, we were using a robot to purify templates on columns, which produced one to two 96-well plates of templates per day at a cost of about $1.50 per template. Although we still use this method for some special applications, we have switched our daily template preparation to an alternate procedure (Mardis, Nucleic Acids Res. 22: 2173 [1994]). This procedure costs about $0.05 to $0.10 per template, and one person can readily do two to four 96-well plates per day. This change alone, which saves us about $1.00 per template, will save us about $75,000–100,000 in supply costs per year given our current sequencing rate of 1500–2000 templates per week.

This new procedure, like all of those we incorporate, was first tested on a limited scale to resolve problems, and then additional members of the group were trained in the procedure in order to put it into general use. We knew that the use of this template procedure might also require a change in some of our sequencing reaction chemistry. New dye, labeled primers, and a new thermostable polymerase became available in 1996 which, in theory, would enable us to increase our sequencing efficiency and would cost less than other commercially available reagents. We implemented this new chemistry in the same gradual way as the template preparation. These reagents save us about $2.50 to $3.00 per reaction over other commercially available reagents. Thus, they provide about the same cost advantage as the homemade reagents we had been using but substantially free us from reagent preparation and quality control issues created by using homemade reagent kits for sequencing. In addition, because these reagents provide superior results, this has allowed us to greatly reduce the time spent editing and finishing the sequence (which is the most time-consuming part of the sequencing process). As part of this development process, we adapted these new reagents, called energy transfer primers and Thermosequenase, to the Catalyst robot in our lab. We were one of the first to accomplish this and made the procedure available on our World Wide Web site for other researchers.

To meet our sequencing throughput requirements, we implemented a number of new techniques to increase efficiency. Chief among these was switching all of our template production and sequencing reactions to a 96-well format. This caused some difficulty initially since the smaller volumes used make many of the steps in sequencing more subject to difficulties in mixing and pipetting. However, this change has enabled us to use various tools such as 8-, 12-, and 96-channel pipettors for many of the steps in the sequencing process. This saves us considerable time given the large number of pipetting steps necessary in a given week. These tools have enabled us to move from 300–600 reactions per week to about 1500–2000 reactions per week with about a twofold increase in the number of people who actually carry out the daily sequencing reactions. The other personnel added to the group are involved in software development, library construction, and various other tasks. In a similar manner, we began carrying out many of the steps in the sequencing process using reduced reagent volumes. This allowed us to cut costs but initially caused problems because reduced volume reactions are inherently less robust than full volume reactions. During the scale up, our success rates were erratic, but they have now stabilized in the same range as that seen in other large-scale sequencing labs. We are upgrading several of our sequencers in the coming year and anticipate that we will be able to increase our throughput 20–40% with the same number of people while maintaining the same success rates. This will be due to the solid foundation we have built in the past year by tearing down all of our procedures and rebuilding the sequencing process with a higher capacity, higher efficiency target.

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**Genome Sequencing of Arabidopsis thaliana**


We have formed a consortium with colleagues at the Genome Sequencing Center at Washington University (GSC) and Applied Biosystems (ABI) to sequence a large region of the genome of the plant Arabidopsis thaliana. We are also collaborating with the European Scientists Sequencing Arabidopsis (ESSA) to coordinate regions of the genome that we will be sequencing. This project is part of a larger international collaboration to sequence the entire genome of this important model organism. The larger collaboration is responsible for making sure that regions of the genome are allocated to the sequencing groups, such as ours, in an equitable and efficient manner.

Our initial effort was a pilot project to sequence a cosmid clone which we selected and which contained the Prolifera gene from chromosome IV (Springer et
FIGURE 1 Predicted genes found in Arabidopsis cosmid AGAA. Predicted genes are shown as blocks placed on the backbone representing the sequence of the cosmid. Numbers on the backbone represent distances in kilobases.

FIGURE 2 Predicted genes in Arabidopsis BAC clone 10P11. Predicted genes are shown as blocks on the line representing the sequence. Numbers on the line represent coordinate positions on the BACs measured in kilobases. The region is one contiguous sequence; it is shown on multiple lines for ease of display.
This clone was completely sequenced within the past year. Figure 1 shows the genes identified in this cosmid. We also worked with our collaborators at the GSC and ABI to sequence a bacterial artificial chromosome (BAC) clone from the short arm of chromosome IV that was provided to us by Ian Bancroft of ESSA. BAC clones contain substantially more insert DNA than do cosmid clones (two to five times as much typically), and we are switching our sequencing to BACs rather than cosmids to increase our efficiency. It did, however, take some time to learn protocols to deal with the larger clones. This BAC clone (~115 kb) was nearly completed in the past year. Two small, specific regions of this clone have proven to be very difficult to sequence, but the remainder of the clone has been completed. Figure 2 shows a diagram of the genes found within this clone.

To scale up our Arabidopsis sequencing program, we began a major mapping effort. Scientists at the John Innes Center determined a contig map of yeast artificial chromosomes (YACs). We used these clones to probe by hybridization high-density BAC arrays and to select the BACs from those areas of the genome. Once BACs are placed in "bins" based on their YAC hybridization data, our colleagues at the GSC employ their high-capacity restriction digest fingerprinting technology to align them with one another and determine a "tiling path" of minimally overlapping clones. The minimal BAC tiling path is the sequencing substrate. This mapping has provided sequencing targets sufficient for our needs until near the end of 1997.

After obtaining these additional clones, we have begun the sequencing of some of them. The shotgun portion of the sequencing of one of these BACs (10M13) was finished during 1996. With our mapped clones and sequencing infrastructure in place, our 1997 target is to sequence 1–1.5 Mb of Arabidopsis. Our fellow consortium members should combine to sequence about the same amount based on the mapped, sequence-ready templates; we are jointly developing for this project. We are releasing our Arabidopsis sequencing data to the community in a very rapid manner. We are also releasing preliminary data through our World Wide Web site. This site can be found at http://www.cshl.org/genseq. It will also have the latest information on other aspects of the Arabidopsis mapping and sequencing projects.

PROTEIN CHEMISTRY

R. Kobayashi  H. Cai  J. Kahler  C. Kelley
P. Kearney  G.J. Salas  G. Binns
N. Carpino  N. Poppito  K. Wanat

Jennifer Kahler, Hui-Zhi Cai, and Camille Kelley left our group this year, and Kimberly Wanat became a new member. This summer, G. Jessica Salas spent 10 weeks with us as an URP (Undergraduate Research Program). Two highlights of the year are that Nick Carpino cloned a gene encoding GAP-associated p62 and we purchased a matrix-assisted laser desorption ionization time-of-flight mass spectrometer funded by a shared instrumentation grant from the National Institutes of Health (NIH).

GAP-associated p62 in Chronic Myelogenous Leukemia

N. Carpino, J. Kahler, B. Stillman, R. Kobayashi
[in collaboration with B. Clarkson, Memorial Sloan-Kettering Cancer Center]

Characteristic of chronic myelogenous leukemia (CML) is the presence of the chimeric p210\textsuperscript{bcr-abl} protein possessing elevated protein tyrosine kinase activity relative to normal c-Abl tyrosine kinase. Hematopoietic progenitors isolated from CML patients in the chronic phase contain a constitutively tyrosine phosphorylated protein that migrates at 62 kD by SDS-PAGE and associates with the p120 Ras GTPase-activating protein (GAP). We have purified p62\textsuperscript{dok} from a hematopoietic cell line expressing p210\textsuperscript{bcr-abl}. p62\textsuperscript{dok} is a novel protein with features of a signaling molecule. Association of p62\textsuperscript{dok} with GAP correlates with its tyrosine phosphorylation. p62\textsuperscript{dok} is rapidly tyrosine phosphorylated upon activation of the c-Kit receptor, implicating it as a component of a signal transduction pathway downstream from receptor tyrosine kinases.
Protein Chemistry
Core Facility

C. Kelley, N. Poppito, K. Wanat, G. Binns, R. Kobayashi

Collaboration with other scientists at Cold Spring Harbor Laboratory continued as a major activity in our laboratory. This year, we purchased an additional piece of equipment to study protein structure (especially posttranslational modifications). An NIH shared instrumentation grant was submitted in March 1995 and funded this year. Contributions are Ryuji Kobayashi, David Beach, Adrian Krainer, Bruce Futcher, Yuri Lazebnik, Carol Greider, Mike Mathews, Sha Mi, Nouria Hernandez, Bruce Stillman, Winship Herr, and Nicholas Tonks. The instrument, a matrix-assisted laser desorption ionization time-of-flight mass spectrometer, arrived at Cold Spring Harbor in October 1996. It is capable of analyzing the molecular weight of peptides as low as 50 femtols. With this instrument, we have been able to identify in vivo phosphorylation sites by analyzing peptide fragments separated by high-performance liquid chromatography (HPLC) to obtain partial amino acid sequences of purified protein. This state-of-the-art instrument has added a significant capability for structural analysis of protein to the laboratory, and its utility has been growing as we spend more time this instrument.

In-gel Digestion for Internal Sequence Analysis of Protein Stained by Silver

H. Cai, N. Poppito, R. Kobayashi

Since internal sequence analysis of protein has an essential role in a strategy of cloning a specific gene, this technique has been extensively used at Cold Spring Harbor since 1992. The method has been improved further and optimized for protein stained with silver. Our intensive study showed that the sensitivity limit of our current in-gel digestion technique is 2 pmoles when we applied the technique on carboxylic anhydrase. Detailed conditions and method will be published in 1997.

PUBLICATIONS


In Press


Faleiro, L., R. Kobayashi, H. Fearnhead, and Y. Lazebnik. 1997. Multiple species of CPP32 and Mch2 are the major active caspases present in apoptotic cells. EMBO J. (in press).


MACROMOLECULAR CRYSTALLOGRAPHY

X. Cheng
A. Dong, W. Gong, J. Horton
L. Jokhan, Y. Liu, T. Malone
K. McClay, M. O'Gara, R. Xu
X. Zhang

Our goal is to determine the structures of a number of biologically important proteins, to atomic resolutions, for a better understanding of their functions in cellular processes.

Within the year of 1996, Karen McClay, a technician who was a part of the group for 4 years, left the laboratory. Ruiming Xu was recognized with a promotion to assistant investigator. Ruiming will continue to study the structure of UP1 and other related proteins and we wish him all the best. Lana Jokhan obtained her master's degree and accepted a position at Bristol-Myers Squibb. Two new postdoctoral researchers, Xujia Zhang and Weimin Gong, and one visiting scientist, Aiping Dong, joined the laboratory.

DNA Methylation

X. Cheng

DNA methyltransferases (Mtases) are found in organisms ranging from archaea and bacteria to plants and mammals. In many of the archaea and bacteria, these enzymes are components of restriction-modification systems. Some Mtases have roles in many eukaryotic cell processes including control of transcription, genomic imprinting, mutagenesis, DNA repair, and development. The DNA Mtases transfer a methyl group from S-adenosyl-L-methionine (AdoMet) to a given position of a particular DNA base within a specific DNA sequence. These Mtases belong to two families: one (including M·Hhal) methylates the C5 ring carbon of cytosine yielding 5-methylcytosine (5mC), whereas the second family (including M·PvuII and Dam) methylates the exocyclic amino group of cytosine or adenine yielding either N4-methylcytosine (N4mC) or N6-methyladenine (N6mA).

Rotation of a DNA nucleotide out of the double helix and into a protein-binding pocket ("base flipping") was first found to occur with Hhal DNA Mtase (Klimasauskas et al., Cell 76: 357 [1994]). There is now evidence that a variety of proteins use base flipping in their interactions with DNA. Although the mechanism for base flipping is still not clear because no intermediates have been character-
ized, we propose a three-step pathway: recognizing the target site and increasing the interstrand phosphate-phosphate distance nearby, initiating the base flipping by protein invasion of the DNA, and trapping the flipped DNA structure (Cheng and Blumenthal 1996). Our laboratory is focusing on finding a basis for flipping bases.

A Structural Basis for the Preferential Binding of Hemimethylated DNA by Hhal Mtase

M. O'Gara [in collaboration with R.J. Roberts, New England Biolabs, Inc.]

M·Hhal, a 5mC Mtase, has been structurally characterized in complex with its DNA substrate in three different methylation states: unmethylated, hemimethylated, and fully methylated. This year, the crystal structure of M·Hhal complexed with nonpalindromic duplex DNA containing a hemimethylated recognition sequence, and with the cofactor analog S-adenosyl-L-homocysteine (AdoHcy), has been determined. The structure provides an explanation for the stronger affinities of DNA Mtases for hemimethylated DNA than for unmethylated or fully methylated DNA in the presence of AdoHcy.

The unmethylated target 2′-deoxycytidine flips out of the DNA helix, and the CH group at position 5 makes van der Waals contacts with the sulfur atom of AdoHcy. Selectivity/preference for hemimethylated over fully methylated DNA may thus reflect interactions among the chemical substituents (H or CH₃) at the C5 position of the flipped cytosine, protein, and the bound AdoHcy. The 5-methyl-2′-deoxycytidine on the complementary strand remains in the DNA helix, with the methyl group almost perpendicular to the carboxylate group of Glu-239, which is part of the sequence-recognition loop. Thus, selectivity/preference for hemimethylated over unmethylated DNA appears to result largely from van der Waals contacts between the planar Glu-239 carboxylate and the methyl group of the 5-methyl-2′-deoxycytidine.

Furthermore, the positive electrostatic potential originating from the bound AdoHcy extends to the DNA phosphate groups flanking the flipped cytosine. The increased binding to DNA by long-range electrostatic interactions should also occur with the methyl donor AdoMet.

Selenomethionyl PvuII Mtase

M. O'Gara [in collaboration with R.M. Blumenthal, Medical College of Ohio]

We have a much more limited understanding of the amino-Mtases (N6mA and N4mC). N4mC Mtases were discovered only relatively recently, and to date the gene sequences of fewer than a dozen have been determined (in contrast to ~45 5mC and ~35 N6mA Mtases). M·PvuII was the first N4mC Mtase for which the nucleotide sequence of the gene and the crystal structure (see below) were determined. The Mtase methylates the internal cytosine of CAGCTG sequences.

The method of multiwavelength anomalous diffraction (MAD) of X-ray synchrotron radiation is now routinely used to solve crystal structures. MAD experiments demand the presence of a few anomalous scatterers at fixed positions. We chose to incorporate selenium (Se) atoms as the anomalous scatterers. The standard approach to production of a selenomethionine (SeMet) derivative protein is to express the protein in strains auxotrophic for Met. Expression of a SeMet derivative protein in such a strain generally increases the chance for complete incorporation of SeMet. However, the M·PvuII-coding plasmid was not moved into a strain auxotrophic for methionine because we did not have an auxotrophic strain that was MrBC−; expression of M·PvuII is lethal unless the host strain is MrBC−. Instead, we efficiently incorporated SeMet into M·PvuII, overexpressed in an Escherichia coli methionine prototrophic strain, DH10B. To assess the level of SeMet incorporation, mass spectrometry was carried out on both intact and digested selenomethionyl M·PvuII, in collaboration with R. Kobayashi (CSHL).

Mass spectrometry analysis of selenomethionyl M·PvuII revealed three major forms that probably differ in the degree of SeMet incorporation and the extent of SeMet oxidation. Amino acid sequencing, mass spectrometry analysis of SeMet-containing peptides (products of a protease digestion), and crystallographic analysis (see below) suggest that all nine methionines have been substituted by SeMet; at least three (Met-30, Met-51, and Met-261) were partially replaced and one (amino acid 261) was preferentially oxidized.

Selenomethionyl M·PvuII was crystallized as a binary complex of the methyl donor AdoMet in the monoclinic space group P2₁. Two complexes were present per asymmetric unit. The crystal, frozen at
95°K, used for the MAD experiment diffracted to 3.3 Å resolution. The MAD data were collected at three different wavelengths corresponding to the inflection point λ1 and the peak λ2 of the Se-containing crystal absorption spectrum, and a third wavelength (λ3) at an energy of 852 eV above the peak position. The anomalous and isomorphous difference Patterson maps at the Harker section (v = 1/2) among data sets collected at wavelengths λ1, λ2, and λ3 showed a number of peaks corresponding to possible Se sites and cross-vectors.

The incorporation of SeMet in place of methionine residues provides a general vehicle for phase determination as well as for polypeptide chain tracing. The MPvull (amino acids 14-336) has nine methionines out of 323 amino acids (2.8%), greater than the 1.7% of averaged occurrence of methionine in proteins. On the other hand, the presence of many Se sites poses an exciting challenge for locating their positions and for MAD applications.

**Structure of Pvull Mtase-AdoMet Complex**

W. Gong, M. O’Gara [in collaboration with R.M. Blumenthal, Medical College of Ohio]

There are a total of 18 possible Se sites per asymmetric unit (9 per molecule). Thus far, a total of 12 sites (6 per molecule) have been manually determined from Patterson maps and confirmed by the twofold noncrystallographic symmetry operator. The geometrical relationship of the 12 Se atoms showed a noncrystallographic twofold symmetry and this local symmetry was consistent with the result of the self-rotation function calculated between the two MPvull molecules in the asymmetric unit. Among the other three SeMet residues whose Se positions were not determined, SeMet\(^{14}\) (the amino-terminal methionine) and SeMet\(^{184}\) are located in disordered regions, whereas the side chain of SeMet\(^{241}\) is flexible.

These 12 Se positions were used for MAD phases, with a final figure of merit of 0.58 at 3.3 Å resolution, using λ1-λ3 and λ2-λ3 isomorphous differences and both λ2 and λ3 anomalous dispersions. The phases were improved by solvent fluffeting and twofold molecular averaging. The initial MPvull model was built at 3.3 Å resolution and the final model was refined at 2.8 Å resolution with the crystallographic R-factor of 0.19.

Structurally, MPvull is folded into two broad domains: a larger catalytic domain that contains the active site and AdoMet-binding regions, and a smaller DNA-recognition region (Fig. 1). The two domains are connected by a flexible linker in the absence of DNA. The catalytic domain shares the "consensus" structural characteristics defined by the SMC Mtase M·HhaI, the N6mA Mtase M·TaqI, and a small molecule AdoMet-dependent Mtase.

**Bacteriophage T4 DNA Adenine Mtase (Dam)**

X. Zhang [in collaboration with S. Hattman, University of Rochester]

Most of the known bacterial DNA Mtases are part of restriction-modification systems, but there are some, such as Dam, that have no corresponding cognate endonuclease. Dam methylation is the only documented case of prokaryotic methylation involved in the regulation of cellular processes. Dam methylates the adenine in the sequence GATC, generating N6mA. T4 Dam has a molecular mass of 30.4 kDa, exists as a monomer in solution, and is similar to bacterial type II DNA Mtases with respect to kinetic parameters.

The enzyme has been overexpressed and purified to near homogeneity. The purification was a straightforward process. Bacterial cells were lysed, and the cell debris was removed by centrifugation. The resulting supernatant was loaded onto a DEAE-cellulose (DE52) column, and the flow-through was collected. This solution was then loaded onto a P11 phosphocellulose column, and the protein was eluted with a linear gradient of NaCl. The peak was pooled and then loaded onto a hydroxyapatite column or an FPLC Mono S column. For purification of the Dam-AdoHcy binary complex, AdoHcy was added to the protein to a final concentration of 0.2 mM and the mixture was incubated on ice for 2 hours prior to loading onto the last column. The resulting pure protein-AdoHcy complex was then concentrated.

The availability of highly purified T4 Dam has facilitated our crystallization trials. A series of synthetic oligonucleotides containing the GATC sequence, but varying in length and nature of ends (Table 1), were incubated with equimolar amounts of the purified and concentrated Dam-AdoHcy. The Dam-AdoHcy-DNA ternary complexes were screened for
crystallization by a sparse matrix using vapor diffusion at 16°C with hanging droplets (6 μl). Five of nine complexes yielded crystals, but only two crystals diffracted X-rays to a medium resolution: a 15-mer with an T-overhang (15-TT) and a 12-mer with blunt ends (12-blunt).

The crystals with the 15-TT DNA attained a size of about 0.3-0.4 mm in one day. Preliminary analysis showed that the crystals were of the monoclinic space group C2 and had unit-cell dimensions of a = 146 Å, b = 110 Å, c = 96 Å, and β = 107°, with four complexes per asymmetric unit. The crystals with the 12-blunt DNA exhibited irregular shape and twinning. Microseeding and macroseeding were used to obtain better crystals. The crystals diffracted X-rays to approximately 3.3 Å using flash freezing and were of the P2₁ space group with cell dimensions of a = 40.3 Å, b = 115.7 Å, c = 74.2 Å, and β = 104°. There are two complexes per asymmetric unit. Efforts to determine the structure of T4 Dam bound to this 12-blunt DNA are currently under way, by conventional

### TABLE 1 Oligonucleotides Screened for Cocrystallization with T4 Dam-AdoHcy Complex

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Cocrystals</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-blunt</td>
<td>5'-ACGGATCCGT-3'</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>3'-TGCTTAGGCA-5'</td>
<td></td>
</tr>
<tr>
<td>11-TT</td>
<td>5'-TCAGGATCCTG-3'</td>
<td>long needle shape</td>
</tr>
<tr>
<td></td>
<td>3'-GTCTTAGGACT-5'</td>
<td>diffract &gt;10 Å</td>
</tr>
<tr>
<td>12-blunt</td>
<td>5'-ACAGGATCCCTGT-3'</td>
<td>suitable for X-ray</td>
</tr>
<tr>
<td></td>
<td>3'-GTCTCTAGGACA-5'</td>
<td>diffract &gt;3.3 Å</td>
</tr>
<tr>
<td>13-TT</td>
<td>5'-TCACGGATCCTGTG-3'</td>
<td>big, good shape</td>
</tr>
<tr>
<td></td>
<td>3'-GTCTCTAGGACT-5'</td>
<td>diffract &gt;10 Å</td>
</tr>
<tr>
<td>13-AA</td>
<td>5'-AACAGGATCCCTGT-3'</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>3'-GTCTCTAGGACA-5'</td>
<td></td>
</tr>
<tr>
<td>14-blunt</td>
<td>5'-CACAGGATCCTGTG-3'</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>3'-GTCTCTAGGACAC-5'</td>
<td></td>
</tr>
<tr>
<td>15-TT</td>
<td>5'-TCACAGGATCCTGTG-3'</td>
<td>big, good shape</td>
</tr>
<tr>
<td></td>
<td>3'-GTCTCTAGGACACT-5'</td>
<td>diffract &gt;4.5 Å</td>
</tr>
<tr>
<td>15-AA</td>
<td>5'-ACACAGGATCCTGTG-3'</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>3'-GTCTCTAGGACACA-5'</td>
<td></td>
</tr>
<tr>
<td>16-blunt</td>
<td>5'-TCACAGGATCCTGTA-3'</td>
<td>big, good shape</td>
</tr>
<tr>
<td></td>
<td>3'-AGTGCTCTAGGACACT-5'</td>
<td>diffract &gt;6 Å</td>
</tr>
</tbody>
</table>

The recognition sequence for Dam is in bold and underlined.
heavy-atom methods (soaking the crystal in the solution of mercury methyl chloride or using iodinated nucleic acid bases) and the MAD method of brominated nucleic acid bases.

**PvuII Endonuclease**


No discussion of restriction-modification systems would be complete without considering their partners, the DNA restriction enzymes or endonucleases. Several years ago, this laboratory determined the structure of PvuII endonuclease (R·PvuII) with cognate DNA (Cheng et al., *EMBO J.* 13: 3927 [1994]). This type II restriction endonuclease from the organism Proteus vulgaris cleaves DNA between the central GC of its recognition sequence (5' -CAGCTG-3' ) in an Mg++-dependent reaction, generating blunt-ended products. This year, two new R·PvuII structures are of peculiar interest: R·PvuII complexed with iodinate cognate DNA (R·PvuII-iDNA) and a mutant R·PvuII(D34G) complexed with cognate DNA. High-resolution data were collected from crystals of these complexes at 1.78 and 1.59 Å, respectively. In studies of PvuII(D34G), where Asp-34 is replaced by a glycine, the protein binds to the canonical DNA sequence with the same affinity as the wild-type but is catalytically deficient. Previously, the structure of the wild-type protein showed that Asp-34 was involved in DNA recognition through minor-groove contacts. Now it appears that Asp-34 must be involved with catalysis as well.

Our high-resolution R·PvuII(D34G)-DNA structure shows clearly that it is not the conformation of the protein that changes but that of the DNA. In particular, the DNA conformation is much different where the R·PvuII cuts the DNA as compared to the wild-type R·PvuII-DNA structure. In the R·PvuII (D34G)-DNA complex, this local region takes on more of an A-DNA conformation than a B-DNA conformation. Such differences in conformation of a local region can alter the phosphate backbone surface of the oligonucleotide and thereby position the target scissile bond of the phosphate backbone differently.

Often, the importance of water molecules in macromolecular recognition is overlooked. High-resolution macromolecular structures yield much information about ordered water structure and corroborate its significance in this process. Fortunately, we can compare, at comparable resolutions, the ordered water molecules in the structures of R·PvuII (D34G)-DNA and R·PvuII-iDNA, where the C of the central GC is iodinated at the N4 position in the major groove and approximates the methylated DNA. Our structure comparison shows that an extensive, well-ordered water network does not exist in R·PvuII-iDNA as it does in R·PvuII(D34G)-DNA. Additionally, it appears that two histidine residues (His-84 and His-85) have two discrete conformations in the R·PvuII-iDNA structure. We surmise that these two His residues are locked into position through recognition of the well-ordered water network found in the unmethylated recognition sequence and are primed to aid in efficient catalysis. Interestingly, in a recent structure-function study by Nastri and Riggs, replacement of His-84 with leucine resulted in a mutant protein that had 1/100 of the specific activity of the wild-type protein but bound with nearly the same affinity. Mutants of His-85 bound DNA with high affinity but their catalysis activity was seriously compromised or totally abolished. Perhaps the DNA "appeared" methylated to these mutants and catalysis could not efficiently be established.

**VP16-induced Complex**

Y. Liu [in collaboration with C. Huang and W. Herr, Cold Spring Harbor Laboratory]

Last year, we reported the crystallization of the central core region (amino acids 48-412) of VP16, which is sufficient for VP16-induced complex formation but lacks the activation domain, and MIR phases from two mercury derivatives. This year, we are using an experimental approach to improve the accuracy of the phases: the MAD method using SeMet and pCMBS-derivative crystals. We have collected Se-MAD data (at two wavelengths) and Hg-MAD data (at two wavelengths) at the National Synchrotron Light Source, Brookhaven National Laboratory, using beamline X12-C.

VP16<sub>48-412</sub> contains ten methionines. We first transformed a Met-autotrophic *E. coli* strain (DL41) with the VP16 expression construct and then overexpressed VP16<sub>48-412</sub> protein in medium that supplies SeMet to obtain the Se-containing proteins. The purification procedures and crystallization conditions of Se-containing VP16<sub>48-412</sub> are the same as those for
the native VP1648-412. Incorporation of the Se atoms in the crystals was monitored by assaying the Se absorption edge through X-ray fluorescence measurements. The Se atoms will also help us to trace the sequence. In addition, we have collected two-wavelength data from a pCMBS-soaked crystal (3 mm for 26 hours) at near the Hg L\textsubscript{III} absorption edge. The anomalous difference Patterson maps show strong peaks corresponding to the three pCMBS sites. Experiments are under way to improve the interpretability of the electron density map.

UP1

R. Xu, L. Jokhan [in collaboration with A. Mayeda and A. Krainer, Cold Spring Harbor Laboratory]

UP1, the amino-terminal domain of A1 heterogeneous nuclear ribonucleoprotein (hnRNP A1), comprises two tandem RNA-recognition motifs. Both motifs are necessary for efficient RNA binding and for the alternative splicing activity of hnRNP A1. Human UP1 has been overexpressed in *E. coli*, purified to near homogeneity, and crystallized in space group P2\textsubscript{1} with unit cell dimensions of \(a = 37.94\ \text{Å}, b = 43.98\ \text{Å}, c = 55.64\ \text{Å}, \) and \(\beta = 93.9^\circ\). The crystal diffracts X-rays strongly beyond 1.3 Å resolution and a native data set to 2.0 Å has been completed. Diffraction data sets from platinum- and mercury-derivatized crystals have also been collected. The three-dimensional structure of UP1 is presented in Dr. Xu’s section.

Beamline X26-C at the National Synchrotron Light Source, Brookhaven National Laboratory

J. Horton [in collaboration with M. Capel and P. Siddons, Brookhaven National Laboratory]

Increasingly, it is becoming essential for biologists to have easy access to a synchrotron beamline in order to be successful in obtaining three-dimensional information for macromolecular structures. Synchrotron light sources provide X-rays that can be 10,000 times as intense as CSHL’s X-ray generator. This intensity allows better data to be collected not only faster, but also from smaller crystals and usually to higher resolution. Another advantage of synchrotron light is that the experimenter can fine-tune its wavelength; this allows for quicker solving of the "phase problem" of crystallography.

In collaboration with the State University of New York at Stony Brook (SUNY-SB) and the Biology Department at Brookhaven National Laboratory (BNL-Biology), we are converting beamline X26-C at the National Synchrotron Light Source (NSLS) at BNL for use in routine X-ray diffraction data collection from crystals of macromolecules. Previously, this beamline had been used for the development and application of X-ray microscopy techniques and for Laue diffraction protein crystallography.

During the past year, we have acquired the necessary equipment and have begun assembly of a configuration that will offer CSHL structural biologists excellent opportunities for data acquisition. We are well under way to satisfying our immediate goals of readying X26-C for our data collection requirements. First, a new monochromator has been installed by the NSLS staff (under P. Siddons) and the beamline has been modified to accommodate it. This monochromator will have a high-energy resolution and a wide energy range so that almost any usable X-ray wavelength can adequately and accurately be reached.

Second, several pieces of equipment have been purchased, adjusted, and tested in the past year: a 300-mm MAR Research imaging plate for data collection; two Silicon Graphics computers for data reduction; 22 gigabytes of disk storage; an 8-mm tape subsystem for data backup; a PC workstation for manipulation and monitoring of beamline hardware; an Oxford Cryostream liquid-nitrogen cryostat for freezing crystals during data collection; one working and another large storage liquid nitrogen dewar for the cryostat; and apparatus for automatic filling of the working dewar. Much of this apparatus has been modified for synchrotron use by the BNL-Biology team (under M. Capel). This equipment has been tested and used on beamline X25 by several experienced crystallography groups, including CSHL’s groups (Cheng, Joshua-Tor, and Xu).

In the coming year, we plan to construct a MAR/FAST hybrid diffractometer and install the BNL-Biology software already written for a similar hybrid at the X12-C beamline. These two steps will allow us to align crystals accurately and routinely in various orientations for measuring anomalous diffraction used in phase determination. Additionally, we will establish and document the data-collection procedures peculiar to the new configuration of X26-C.
When completed, X26-C should be as productive or more productive than other beamlines dedicated to macromolecular crystallography at NSLS.

**PUBLICATIONS**


In Press


**STRUCTURAL BIOLOGY**

L. Joshua-Tor  T. Messick  J. Rosenbaum

Our focus is to study the molecular basis for regulatory processes in terms of molecular recognition. We are using tools of structural biology and biochemistry in a combined approach to look at proteins and protein complexes associated with these processes. X-ray crystallography, our primary technique, enables us to get a very accurate three-dimensional structure of our proteins and the interactions in which they are involved. We use biochemistry to study properties predicted by the structure and to direct our structural studies in an iterative manner. We are also combining information from molecular biology and genetics in collaborative efforts to study their functions. Our current efforts center around two distinct themes: (1) the study of an evolutionarily conserved family of oligomeric intracellular proteases, the bleomycin hydrolases, and (2) structural studies of transcription factor complexes. Here, we are currently screening crystallization conditions.

**THE BLEOMYCIN HYDROLASES**

Resistance to antineoplastic drugs, whether intrinsic or acquired, is a central problem in treatment of human cancers. Bleomycin hydrolase is a cysteine protease discovered by its ability to hydrolyze the anticancer drug bleomycin and thus to limit its use in cancer therapy. The recent discovery of homologs of this intracellular hexameric 300-kD protein in yeast and bacteria implies a conserved cellular function for this protein. In humans, this protease is expressed in all tissues tested and at elevated levels in several tumors. In mice, expression is highest for newborn mice and decreases with age. The crystal structure of the yeast bleomycin hydrolase, Gal6, which we have determined, has revealed several unique features of these proteases. These include a ring structure with a central channel where the six active sites can be accessed only from within the channel, a nucleic-acid-binding activity, and an unusual positioning of the completely conserved carboxyl terminus of the protein in its own active site cleft. The yeast protein binds preferentially to single-stranded DNA or RNA with a \( K_d \) of 10 nm and to nicks in double-stranded DNA. Moreover, its expression is regulated by galactose and the Gal4 protein, and Gal6 itself acts as a repressor of galactose-regulated genes. Clearly, bleo- mycin hydrolase has complex cellular functions, consistent with the increasing recognition of the important role in general of intracellular proteases.
The Carboxyl Terminus of Gal6, the Yeast Bleomycin Hydrolase, a Molecular Ruler for Proteolysis

L. Joshua-Tor [in collaboration with W. Zheng and S.A. Johnston, Southwestern Medical Center]

One of the more interesting features of this enzyme is the carboxy-terminal arm and its role in proteolysis. The carboxy-terminal arm of Gal6 extends into its own active site cleft and structurally resembles peptide inhibitors of papain. In this way, the carboxyl terminus may serve as a regulator of enzyme activity. The sequence is completely conserved in this region (see Fig. 1) apart for the very last residues. We have shown that for the yeast and human homologs, these unconserved residues appear to be removed in the...
mature enzyme, most likely by an autocleavage process, both of them terminating with an alanine as the last residue of the polypeptide chain. In fact, if we mutate the active site cysteine to an alanine in the yeast enzyme, the terminal lysine is not removed and is well ordered in the crystal structure we have solved for this mutant (see below).

The positioning of the carboxy-terminal arm in the active site cleft explains its amino peptidase activity in vitro. On the basis of crystal structure of the yeast enzyme, we propose that the substrate is positioned such that its amino end is held by the carboxylate of the carboxy-terminal arm in position for cleavage. The geometry would be such that the amino-terminal residue would be cleaved. This is consistent with the observation that N-blocked peptides are not cleaved by these enzymes. To test this hypothesis, several mutants were prepared with various truncations at the carboxyl terminus. We have shown that we can turn the enzyme into an endopeptidase, cleaving a peptide substrate at a predicted position. Thus, the carboxy terminus acts as a molecular ruler, resulting in an enzyme that has positional specificity, rather than sequence specificity. This is also consistent with the observation that these enzymes have a rather broad substrate sequence specificity.

A Hinge in the Carboxy-terminal Arm of Gal6 Controls Autoproteolysis

L. Joshua-Tor, J. Rosenbaum [in collaboration with W. Zheng and S. A. Johnston, Southwestern Medical Center]

We are currently expressing these proteins as histidine-tagged constructs in Escherichia coli. In this manner, relatively high yields of purified protein can be obtained in a two-step purification, and although we have a TEV protease site, which would enable us to remove the tag, we did not have to cleave the histidine tag even for crystallization purposes. The crystal structures of two mutants of the yeast enzyme have been solved. In both mutants, the active site cysteine was replaced with an alanine. In the first mutant, which was solved at 2.05 Å resolution, the carboxy-terminal lysine was retained. This is in effect a natural substrate for the protease since the enzyme normally cleaves this residue off. In the second mutant, which was solved at even higher resolution, 1.87 Å, this lysine was intentionally removed as it is in the wild-type protein. Thus, a double mutant had to be prepared to achieve the single effective change compared with the wild-type protease. The quality of the electron density maps obtained from these mutants, the latter in particular, is such that even part of the tag is observed in these crystal structures.

When comparing the two mutants with the wild-type protein, a hinge is revealed in the carboxy-terminal arm that appears to control the initial autocleavage reaction necessary for obtaining the mature protein. Most proteases have a site designed to bind a negatively charged oxygen (an oxyanion) which is present in the intermediate of the proteolytic cleavage reaction (termed the "oxyanion hole"). A carboxylate oxygen, being such a moiety, would naturally occupy that site if it could reach it. The effect of removing the sulfhydryl (SH) group from the active site cysteine by substituting it with an alanine is that a path is open for the carboxylate of the carboxyl terminus to reach the "oxyanion hole." One of the carboxylate oxygens in both mutants is sitting in that oxyanion hole. For the second mutant, where the only effective difference with the wild-type protein is a substitution of the sulfhydryl group, this is exactly the location of that group during the initial cleavage reaction. From the superposition of this mutant with the wild-type protein (see Fig. 1), it is clear that the carboxy-terminal arm must move toward the active site in order to occupy that site. To achieve this motion, a "bulge" in the polypeptide chain is straightened out in what would resemble a hinge motion, with a conserved glycine residue (G450) at the hinge (see Fig. 1). A close examination of the angles involved would predict that a glycine would be the only amino acid that would allow this hinge motion.

The Human Bleomycin Hydrolase

L. Joshua-Tor, J. Rosenbaum [in collaboration with H. Whelan and S. A. Johnston, Southwestern Medical Center]

One of our goals is to understand the bleomycin hydrolase activity of the enzyme which leads to drug resistance. The human homolog is the more appropriate target to study in this case, both as the free protein and in complex with the drug. This could also be beneficial in rational drug design for specific inhibitors that would block the bleomycin hydrolase activity and possibly overcome drug resistance and thus
the efficacy of bleomycin as an anticancer agent. Using an expression system in *E. coli* similar to the one we used for the yeast homolog and mutants, the recombinant human homolog is obtained in reasonable yields and purified in a two-step procedure. We were successful in our crystallization attempts and are currently in the process of refining these conditions.

**ENCODED COMBINATORIAL LIBRARIES**

H.P. Nestler  K. Charlston (Student)  R. Liu  
D.L. Dong  R. Sherlock  
J. Montagu (URP)  J. Steiger (PFF)

Encoded combinatorial "One bead–One Structure" libraries have opened a new dimension for the study of intermolecular interactions of low-molecular-weight synthetic receptors with chemical and biological ligands. We are employing combinatorial libraries to identify molecules that specifically interfere with the biological function of proteins of physiological importance at specific functional sites. Hereby, we pursue two different approaches: the search for selective enzyme inhibitors that deactivate enzymes by binding to their active site, and the quest for molecules that bind proteins at specific epitopes on their surface and block the access of other proteins interacting with these epitopes.

**Molecular Forceps from Combinatorial Libraries That Bind RAS Proteins**

D.L. Dong, R. Liu, R. Sherlock [in collaboration with M.H. Wigler, Cold Spring Harbor Laboratory]

Our initial studies have shown that simple molecules displaying two or more short peptide or peptide-like chains (which we termed "molecular forceps") can provide reasonable affinities and selectivities toward peptide substrates. We are using libraries of molecular forceps to identify molecules that are able to bind to the carboxyl terminus of RAS proteins (also termed "CaaX-box") and prevent its farnesylation. The farnesylation of RAS has been shown to be a crucial step in the activation of RAS as a signal transduction protein, and pharmaceutical trials have shown that molecules that inhibit the farnesyl transferase, the farnesylating enzyme, are able to stop tumor growth. The approach we follow outlines a new paradigm for molecular biology, as we will attempt to prevent a biological process not by inhibiting the enzyme connected with this process but by masking the substrate with a synthetic molecule. To efficiently target our molecular forceps toward the carboxyl terminus of RAS, we use the isolated peptide sequence instead of the whole protein as substrate for the screening of our libraries. The molecules selected against the peptide epitope should bind RAS site-specifically at the CaaX-box. Although there is precedence that antibodies can be raised against peptide epitopes in this fashion, it is unclear whether this approach is suitable for the selection of synthetic molecules. The carboxyl terminus of RAS is a suitable test case for this novel approach as it is conformationally not restricted and should therefore be readily accessible for the molecular forceps we intend to generate.

During the past 12 months, we generated a library that contained two-armed and four-armed molecular forceps. When we screened the library against the octapeptide derived from the CaaX-box of RAS, the molecular forceps with four arms, although more flexible than the two-armed forceps, predominated as interacting molecules. This result suggests to us that we will be able to compensate reduced molecular rigidity by increasing the molecular size of our forceps. We could show that the molecules selected for their binding toward the octapeptide also bind the whole protein, establishing that we are able—at least in this case—to select molecules that bind the protein by screening a library against the epitope of interest. All of the molecules that we selected, as well as molecules selected against the whole protein, have shown potential to impede the farnesylation of RAS, and we are currently establishing that this retardation
is caused by binding of the forceps to the CaaX-box of RAS and not by coincidental inhibition of the farnesyl transferase.

Combination Libraries
for the Identification of Enzyme Inhibitors


In the past year, we have engaged in the investigation of using two families of amino acid derivatives for the identification of enzyme inhibitors: sulfonamides as potential inhibitors of proteases and phosphotyrosine mimics as inhibitors of protein tyrosine phosphatases (PTPs). Both enzyme families are involved in controlling cell fate and contain many homologous enzymes. To elucidate the effects of each individual member of these families requires that the functionality of each individual enzyme be abolished selectively. Because of the diversity of compounds present in combinatorial libraries, we hope to select molecules that selectively block the action of the targeted enzymes while leaving homologous enzymes unaffected. Such inhibitors will be valuable tools for the dissection of biochemical pathways.

Sulfonamides have found widespread application as antibiotics. They structurally mimic the transition state of peptide hydrolysis, bind to proteolytic enzymes, but are not cleaved. Thus, the assumed mode of biological activity is through inhibition of proteolytic enzymes. Our collaborators in Milan have developed a new class of sulfonamides, $\beta$-amino-sulfon amino acids, that can be used as building blocks for combinatorial libraries. During the last year, we have begun to develop a library containing regular peptides and peptides containing $\beta$-amino-sulfon amino acids to identify inhibitors for proteases. Phosphotyrosine mimics are known as inhibitors for PTPs. We have prepared one phosphotyrosine analog and incorporated it in a peptide and established that this mimic binds to PTP-1B and inhibits its activity. The next step will be to include this phosphotyrosine mimic in a peptide library to select peptide sequences that inhibit PTP-1B selectively in the presence of its homologs.

Detection of His$_6$-tagged Peptides and Proteins on Beads and Protein Gels

R. Liu, H.P. Nestler, J. Steiger

The attachment of a hexa-histidine moiety ("His-tag") to proteins is a standard means to purify these proteins by affinity chromatography over a nickel column, making use of the strong affinity of the His-tag to the nickel bound to the column. We have developed a technique to use the His-tag for the screening of combinatorial libraries. Beyond its ability to complex nickel ions, the His-tag also interacts with other ions of transition metals, such as copper. Copper(II) ions are essential to catalyze the Benzidine Blue reaction, where the colorless substrate benzidine (biphenyl-diamine) is oxidized by hydrogen peroxide to yield a dark blue charge-transfer complex. When applied for the screening of libraries, beads interacting with the His-tagged ligands develop a brown color which increases over time. As the setup of the staining allows for the catalytic amplification of the signal, the described technique allows the detection of very small amounts of proteins as it is required for the screening of combinatorial libraries. The use of the described staining technique is not limited to the screening of combinatorial libraries. We have adapted this staining for the detection of His-tagged proteins in SDS-PAGE gels. As the His-tagged proteins have a higher affinity toward copper than other proteins, engineered and expressed His-tagged proteins are stained predominantly in gels of protein mixtures.

A Two-dimensional, Diagonal SDS-PAGE Technique to Screen for Protease Substrates in Protein Mixtures

H.P. Nestler

Proteases have emerged during the last years as key players in the execution of programmed cell death. Many homologous proteases have been identified and cloned. Yet, to understand the pathways the proteases are involved in, and to elucidate the cellular cascades they trigger, requires the identification of the intracellular substrates processed by each protease. Even the intensive efforts in genome sequencing and
cloning that yield new protein sequences every day do not yield final answers to these questions. The occurrence of a cleavage motif in a protein sequence is not sufficient for proteolytic susceptibility of the protein, as the folding of the proteins may prevent access of the protease to the substrate site. Indeed, many proteins in their native conformation have been shown to be resistant to proteolytic digestion.

Two-dimensional gel electrophoresis combined with in-gel-proteolysis is a novel approach to screen for protease substrates in protein mixtures: A mixture of proteins, i.e., a cellular extract, is separated in a SDS gel and the lanes are excised. The denatured proteins in the gel stripes are renatured and digested with the protease of interest. The electrophoretic development into the second dimension under the same conditions as the first dimension results in undigested proteins appearing in a diagonal line, whereas the products of the proteolytic cleavage separate from the diagonal. The denaturation of the proteins with SDS during the electrophoresis is necessary to ensure the electrrophoretic separation by molecular weight, which is required to achieve the alignment of uncleaved proteins in the diagonal of the twodimensional gel. The sequences of thus identified protease substrates can later be determined by microsequencing.

PUBLICATIONS

In Press

STRUCTURAL BIOLOGY

R.-M. Xu Y. Zhang
L. Jokhan

One of our main areas of interest is to understand the structural basis of protein-RNA interactions using X-ray crystallography. Currently, we focus on the structure determination of proteins involved in pre-mRNA splicing, including hnRNP A1, SF2/ASF, and U2AF65. A common feature of these proteins is that they all contain several copies of RNA-recognition motifs, and synergy between the RRMs is required for stable and specific RNA-binding and for activity. As a first step in understanding the RRM synergy from a structural point of view, we have crystallized and determined the crystal structure of UP1, the twoRRM domain of hnRNP A1.

Structure Determination of UP1

R.-M. Xu, L. Jokhan [in collaboration with Adrian Krainer, Cold Spring Harbor Laboratory]

Nascent transcripts of RNA polymerase II are bound by a group of nuclear proteins, known as heterogeneous nuclear ribonucleoproteins (hnRNPs). hnRNP A1, one of the most abundant hnRNP proteins, has important roles in a number of cellular processes, including packaging of pre-mRNA transcripts in the nucleus, control of gene expression through regulation of alternative 5' splice site selection, and promoting annealing of complementary single-stranded nucleic acids. In vitro experiments showed that it can act as an RNA chaperone to ensure correct folding of biologically active RNAs, such as ribozymes and tRNAs. hnRNP A1 has also been implicated in transporting mature mRNA from the nucleus to the cytoplasm. RNA-protein interactions are the principal mechanism underlying each of these biological effects of hnRNP A1. Therefore, the determination of the structural basis by which hnRNP A1 interacts with RNA is important in understanding each of these biological processes.

Human hnRNP A1 is a 34-kD protein consisting of a single polypeptide chain of 320 amino acids. An amino-terminal proteolytic fragment spanning the first 196 amino acids and known as unwinding protein
UP1 comprises two RNA-recognition motif (RRM) sequences separated by a short linker. The carboxy-terminal region of hnRNP A1 is rich in glycine and includes several RGG repeats, which also constitute an RNA-binding motif. Early studies showed that both the amino-terminal UP1 domain and the carboxy-terminal glycine-rich domain are capable of binding to nucleic acids, whereas the intact protein exhibits more stable and highly cooperative binding, with cooperativity being due to self-association of the carboxy-terminal domain. Both the UP1 domain and the carboxy-terminal domain are required for the alternative splicing activity of hnRNP A1, and within the UP1 domain, mutations in conserved surface residues of either RRM abolish activity, although general RNA binding is not substantially affected. Purified hnRNP A1 binds with high affinity to short RNAs containing one or more copies of the motif UAGGGA/U, although the physiological significance of this high-affinity binding remains controversial.

In the absence of bound RNA, UP1 was crystallized in space group P21 with cell dimensions a = 38.0 Å, b = 43.9 Å, c = 55.8 Å and β = 94.2°. The unit-cell parameters suggest that there is one UP1 molecule per asymmetric unit in the crystal. The crystal structure of UP1 was determined by the classical MIR method to 1.9 Å resolution. The crystal structure of UP1 shows that each RRM independently adopts the characteristic RRM fold, consisting of a four-stranded antiparallel β-pleated sheet and two α helices packed on one side of the β-sheet. The two RRMs in the UP1 structure are in close contact and are positioned with the β sheets facing the same side (Fig. 1).

The most significant and unexpected finding from the UP1 structure is that the two RRMs are held together through two Asp-Arg salt bridges, which

![FIGURE 1 Ribbon diagram showing the UP1 structure. The conserved RNP-2 and RNP-1 submotifs (β, and β2, respectively) are shaded. The disordered portion of the linker connecting the two RRMs is shown in a dark color; the amino terminus starts at Pro-7, and the carboxyl terminus ends at Ser-182. Amino acids involved in inter-RRM salt bridging and the conserved phenylalanines are shown as ball and stick models.](image-url)
result in juxtaposition of the four-stranded β-sheet from each RRM to form an extended RNA-binding surface. The salt bridges observed may partially account for the observed salt sensitivity of RNA binding by both UP1 and hnRNP A1. Since the two salt bridges in the UP1 structure are partially exposed to the solvent, it may be expected that a high-ionic-strength environment could have a strong destabilizing effect on the packing of the RRMs, by disrupting the Arg-Asp electrostatic interactions. Interestingly, it has been observed that salt concentration can influence alternative splicing in vitro, with increasing ionic strength having an opposite effect on alternative 5’ splice site utilization as that of adding hnRNP A1. The activation of proximal 5’ splice sites upon increasing salt concentration is consistent with destabilization of hnRNP A1 binding through disruption of the inter-RRM salt bridges. The specific residues involved in salt bridging between the two RRMs are highly conserved among hnRNP A/B proteins but not in more distantly related RRMs, strongly suggesting that the structural basis for achieving a defined orientation of the two RRMs relative to each other has been phylogenetically conserved.

Although the present structure does not have bound RNA, a great deal can be inferred about the UP1-RNA interactions from the UP1 structure, in combination with previous biochemical studies of hnRNP A1 and UP1 and with findings from structural studies of single RRM-RNA interactions in other RNA-binding proteins. It is known that the RRM β-sheet provides a general RNA-binding platform, and the conserved aromatic residues located in the two central β strands base-stack with single-stranded bases of the bound RNA. These base-stacking interactions are likely to be an evolutionarily conserved mechanism of RRM-RNA interaction. Although the conserved RRM features are crucial for both general and sequence-specific RNA binding, regions outside of the RRM may also have important roles. In the UP1 structure, the linker connecting RRM1 and RRM2 appears to be flexible, and six residues in the middle are disordered. Nevertheless, the ordered residues in the structure place the linker in a position highly likely to be involved in direct RNA interactions. In addition, structural comparisons of UP1 with other RRM structures suggest that the short amino-terminal 310 helix can potentially play a prominent part in interacting with RNA.

Our next step will be to determine the co-crystal structure with RNA, which will provide a precise picture of RNA-protein interactions by hnRNP A1.

PUBLICATIONS

In Press
Synergy abounds—among genes in a genome, among neurons in a brain, and among neuroscientists at CSHL. This past year has witnessed impressive, important advances in our understanding of the biochemical bases of learning and memory and of the mechanistic link between development and behavioral plasticity. Implications of this new insight for the treatment and cure of learning disabilities are direct and immediate.

Malinow and company have begun to elucidate how a cellular form of memory—long-term potentiation (LTP)—comes about. Young hippocampal neurons initially form synapses that are "silent." Once they mature, however, postsynaptic depolarization coincident with presynaptic activity produces an NMDA receptor-mediated calcium influx into the dendrite. Increased levels of calcium activate a calcium-dependent kinase (CAMKII), which presumably mediates the insertion of new AMPA receptors into the postsynaptic membrane. AMPA receptors then respond to presynaptic release of glutamate even when the postsynaptic membrane is not depolarized, thereby yielding an enhancement of synaptic transmission. Amazingly, this same molecular mechanism may be involved in activity-dependent synaptic stabilization during development of the brain. By imaging neural growth in the frog optic tectum, Cline and workers have shown that newly formed synaptic connections are glutamatergic but are unstable in immature neurons. As the neurons mature, however, CAMKII expression increases and, consequently, NMDA receptor-mediated neural activity converts silent synapses to more active ones again via the appearance of AMPA receptors. These enhanced synapses then become more stable. Thus, the conversion of immature, unstable synapses to mature stable synapses appears to underlie both developmental and behavioral plasticity.

How do these immature neurons arise in the developing brain? Enikolopov and colleagues have been studying the effect of nitric oxide (NO)—a gaseous molecule which is produced by nitric oxide synthase—on neural development. They first established in cell culture that NO helps control a developmental switch from proliferation to differentiation of neuronal precursor cells. More recently, they have obtained direct evidence for this developmental role of NO by assessing its function in vivo using both the fruit fly and frog model systems.

Another important aspect of neurodevelopment concerns heritable learning disabilities. In this context, Silva and colleagues have turned their attention to the neurofibromatosis type1 (NF1) gene, mutations in which produce learning disabilities (and other defects) in children. They have shown that mutant mice carrying a knock-out of NF1 also display distinct learning disabilities, thereby establishing a model system with which to study this heritable disease. Cellular studies have suggested that NF1 may function as part of the Ras/Raf signal transduction pathway. Hence, Silva and coworkers also assessed learning in mutant mice carrying a knock-out of the Ras-GRF exchange factor; such mice also have learning defects. While studying cellular mechanisms of synaptic plasticity in Drosophila, Zhong and coworkers discovered that peptidergic stimulation of motor neurons induced both the Ras/Raf pathway and the cAMP pathway. In fact, coincident activation was required to produce changes in synaptic transmission. This observation, combined with results from the Silva group, prompted Zhong's group to study mutant flies carrying disruptions of an NF1 homolog. They discovered these NF1 mutants to be defective in synaptic transmission and associative learning. These results in mice and flies have led both groups to investigate drug treatments that may ameliorate the learning disabilities of NF1 mutants.
This past year, we have been consolidating our work on the role of cAMP signaling in learning and memory. In collaboration with Jerry Yin’s lab, we are developing new transgenic lines and behavioral training protocols to reveal further CREB’s role in long-term memory (LTM) formation. For these experiments, we have used inducible transgenes to manipulate gene expression in time. We also have succeeded in using enhancer-trap lines to manipulate gene expression in space, thereby demonstrating a role for cAMP signaling in an identified anatomical structure of the adult brain. To extend these gene manipulations, we have begun to build a new genetic tool, which will allow us to manipulate gene expression in time and space.

**dCREB2 Activator Is Rate-limiting for Enhanced LTM**

T. Tully, J. Dubnau, B. Svedberg [in collaboration with J. Yin, Cold Spring Harbor Laboratory]

Evidence from other model systems (Aplysia) has suggested that the rate-limiting step in LTM induction is the cAMP-induced translocation of the protein kinase A (PKA) catalytic subunit from the cytoplasm into the nucleus. Whereas this situation, in fact, may obtain during the acquisition phase of associative learning, we considered it unlikely to be the case once acquisition reached an asymptotic high. At such a time, cAMP signaling would be expected to be maximally activated and thus PKA translocation would be well under way. What then is rate-limiting for the induction of LTM after spaced training? We addressed this question by quantifying enhanced LTM in transgenic dCREB2-a (CREB activator) flies that were heat shocked for different lengths of time. We found that enhanced LTM was a direct function of the duration of heat shock. Thus, enhanced LTM induction is rate-limited by the amount of transgenic CREB activator expressed.

We also have begun to search for genes downstream from CREB that are transcriptionally regulated during LTM formation. Parametric experiments have determined that transcriptional changes of ≥150% can be detected via five RNA extracts from adult heads. Both positive and negative controls have been established, with extant genes known to be involved in cellular or behavioral plasticity. Presently, a screen for new genes will begin.

**Identification of New Genes Involved with Associative Learning**

T. Tully, C. Jones, S. Pinto, J. Christensen, G. Bolwig, T. Bridges, J. DeZazzo, K. Velinzon, H. Solomon [in collaboration with K. Broadie, University of Utah]

We continue our molecular genetic characterizations of latheo, linotte, nalyot, golovan, and amnesiac. We are studying various pleiotropic effects of latheo mutations. In collaboration with Kendall Broadie at the University of Utah, we have discovered that Lat is expressed in embryonic blastoderms, in larval neuroblasts, and in presynaptic terminals of the larval neuromuscular junction and that latheo mutants show an induced hyperexcitability at these synapses (preliminary observations made by Yi Zhong, CSHL). We have raised antibodies to Lio and now are characterizing its expression pattern in adult brains. Genetic complementation experiments have established that the nalyot mutation lies in the Adf-1 transcription unit, which encodes a transcription factor. Similarly, the golovan mutations fail to complement other mutations in extra machrochaetae, a negative regulator of proneural transcription factors. Ex-
Experiments are now under way to determine if these new nuclear factors function during adult learning and memory formation.

**Characterization of Extant Mutants in Signal Transduction Pathways**


We have extended our study of cAMP signaling during olfactory associative learning in two important ways. First, we successfully have detected a biochemical defect in mutant PKA-RI adults: Whole-head homogenates show higher than normal baseline levels of PKA activity in the absence of any exogenous cAMP but normal PKA activity in 5 μM cAMP. Although subtle, this biochemical defect is consistent with the notion that the PKA-RI mutation(s) either yields lower than normal levels of RI protein or produces an dominant-negative mutant protein. Evidence from Northern and Western blot analyses suggests both. Second, we have used enhancer-trap technology to limit expression of dominant-negative mutant or wild-type stimulatory subunits of G protein (Gs) to various anatomical regions of the fly brain. When mutant Gs is expressed preferentially in mushroom bodies, olfactory associative learning is completely abolished, with no detectable effects on the transgenic flies’ task-relevant sensorimotor responses. When mutant Gs is expressed in the central complex (another anatomical region of the fly brain that has been implicated in learning), olfactory associative learning is normal. Likewise, this learning is normal when wild-type Gs is expressed in either brain region (Fig. 1). These data demonstrate for the first time that cAMP signaling in mushroom bodies is required for normal olfactory associative learning, a notion originally suggested by the (preferential) expression patterns of dunce, rutabaga, DCO, and RI.

We have also extended our studies of turnip mutants, which were isolated as defective in olfactory learning and are defective in phospholipid-dependent protein kinase (PKC). We have shown that locomotor reactivity is aberrant in turnip flies. Consequently, these mutants show abnormal task-relevant sensorimotor responses, faster than normal habituation to olfactory cues, abnormally low dishabituation, and less dark reactivity. Such effects preclude the notion that turnip (and PKC activity) underlies olfactory associative learning in Drosophila.

We have generated antisera to dNOS and have begun to characterize its pattern of expression in Drosophila. Interestingly, it appears first expressed during embryogenesis in a region of the midline from which neuroblasts later differentiate. We now are trying to disrupt dNOS to characterize the developmental and behavioral effects of hypomorphic and null mutations.

Finally, we have attempted to rescue the memory defect of amnesiac mutations via induced expression of "the Quinn ORF," a putative neuropeptide that M. Feany and W.G. Quinn have reported to be encoded by amnesiac. We see no rescue. Along with DNA sequence data from the 19A region, this observation suggests that the amnesiac transcript may encode a neuropeptide. Thus, we continue efforts to clone the correct gene.
Behavioral Properties of Learning and Memory in Normal and Mutant Flies

T. Tully, J. Connolly, R. Wlodarczyk, K. Velinzon
[in collaboration with D. Yamamoto, Mitsubishi Kasei]

We continue to develop new training protocols to dissect genetically the biobehavioral organization of learning and memory. This year, we have modified our Pavlovian discriminative conditioning procedure to quantify conditioned excitation (produced by pairing an odor with electroshock) versus conditioned inhibition (produced by pairing an odor with "no shock"). Our data reveal properties of conditioned excitation and inhibition similar to those in vertebrates. Moreover, our behavioral analyses indicate that conditioned inhibition is not produced by our discriminative procedure. Finally, we have discovered that conditioned inhibition is normal but that conditioned excitation is reduced in radish mutants. Thus, we have genetically dissected these two opposing forms of associative learning.

We also have developed a new chemosensory learning procedure for larvae. Initial learning levels are similar between larvae subjected to either spaced or massed training. Memory persists for at least 2 days after spaced training, however, but decays within 2 days after massed training. Thus, even fruit fly larvae show this fundamental (and ubiquitous) property of memory formation. With this larval assay, we will be able to study the effects of mutations on learning in immature "flies" and then characterize the development of adult associative learning.

New Genetic Tools

T. Tully, J. Dubnau, A. Wells

Several of the studies mentioned above capitalized on the use of genetic tools in Drosophila that allow us to manipulate gene function in either time (inducible transgenes) or space (enhancer-trap-driven transgenes). Ideally, however, we want to manipulate gene function in time and space. To accomplish this experimental goal, we have begun to develop a new transgene vector that incorporates both enhancer-trap-driven expression and inducible expression.

Another current limitation in fly molecular genetics concerns targeted mutagenesis of cloned (wild-type) genes. The only method currently available is site-selected P element mutagenesis, which is relatively inefficient and labor-intensive. To solve this problem, we are developing a molecular-genetic method to enhance the frequency of homologous recombination in fruit flies. If successful, this approach will allow replacements of wild-type DNA sequence with mutant sequences of various sorts.

Finally, our past efforts to identify new genes involved with learning and memory have been forced to rely on P element mutagenesis, primarily because this approach yields morphologically and molecularly tagged mutations, which then are more easily cloned. P elements do not insert randomly into the genome, however. Best estimates suggest that these transposons likely will disrupt only half of the 15,000 genes in Drosophila. Chemical mutagenesis, in contrast, mutates genes randomly but does not yield morphologically tagged genes. Moreover, this approach usually produces point mutations, which are next to impossible to detect molecularly. To alleviate this problem, we are developing a molecular method to identify point mutations.

PUBLICATIONS


LONG-TERM MEMORY FORMATION IN DROSOPHILA

We are interested in the molecular biology of long-term memory (LTM) formation. The available information, tools, and technologies that can be applied to this problem vary from experimental system to system, with each organism having unique advantages and disadvantages. We are trying to utilize advantageous approaches, or develop complementary ones, to exploit the relative strengths of the Drosophila and mouse systems.

Upstream Activation of CREB

S. Till, H. Zhou, J. Yin

We have initiated a reverse genetic approach to analyze the role of conserved phosphorylation sites in the "P-box" domain of the Drosophila dCREB2 gene. Phosphorylation sites that are conserved between the mammalian cAMP-inducible transcriptional activators (CREB, CREM, and ATF-1) and the fly protein have been mutated to alanine residues. These mutations have been incorporated into both inducible activator (dCREB2-a) and blocker (dCREB2-b) isoforms of the fly gene and transgenic lines have been made and await behavioral testing. Previous work has shown that when flies carrying the wild-type activator transgene are given a single training trial, there is a linear relationship between the amount of induction and the amount of LTM that is formed, until an asymptote is reached. Transgenic flies that are given a subthreshold amount of induction require more spaced training trials to reach this asymptote. To test the effect of mutating a given phosphorylation site on activator function, we will deliver sub-threshold levels of induction followed by spaced or massed training trials. Similarly, mutant blockers will be tested for their effects on preventing LTM formation.

To complement our in vivo analysis of phosphorylation, we have begun to generate phospho-specific antibodies directed against sites in dCREB2 of particular interest. We have two independent antibodies directed against the phosphorylated Ser-133 residue, whose phosphorylation is absolutely necessary, but not sufficient, for CREB activation across all species. This residue has been shown to be a target for a number of different signaling pathways, including protein kinase A, Ca++-calmodulin kinase II and IV, and CREB-kinase, which lies downstream from a neurotrophin-activated MAP kinase cascade. Currently, we are examining this antibody on Western blots and tissue sections in response to physiological, pharmacological, and genetic manipulations that should result in phosphorylation of this residue. We have also begun making a Ser-142 phospho-specific antibody, but we have been hampered by our inability to phosphorylate efficiently the fly CREB protein on this residue in vitro.

We have been using a mammalian Ser-142 phospho-specific antibody to try to trap this species in mammalian cells and hippocampal slices. Since we believe that phosphorylation of this residue is directly or indirectly responsive to CaM kinase II, we are treating cells or slices with physiological stimuli or pharmacological agents that should activate this pathway. Using Western blot analysis of proteins, or immunohistochemistry of cells, we are looking for evidence that this residue can be phosphorylated.

The LTM Cells

J. Wallach, H. Zhou, J. Yin

We are continuing to characterize our transgenic flies which carry CREB-responsive reporter genes. Two
different types of reporters have been made, those that contain complex promoter fragments from putative "downstream" targets of activated CREB (*Drosophila* fos, tyrosine hydroxylase, and CREB) and those that only contain reiterated CREB-binding sites (CREs).

These insulated reporter flies have been examined for their validity in reporting CREB-activated transcription. With the simpler reporters, a 3x wild-type CRE enhancer generates a much stronger signal than a 3x mutant CRE enhancer, showing that the pattern of staining which we see on adult head sections is sensitive to mutations that decrease the binding affinity of the dCREB2 proteins at least 20-fold in vitro. If the dCREB2-b inducible transgene is crossed into the fly that contains the 3x wild-type CRE reporter, expression from this transgene blocks reporter activity, showing that the enhancer can be responsive to fly CREB isoforms. However, neither genetic or pharmacological manipulations have ever resulted in up-regulation of reporter expression. Our attempts to visualize LTM-specific staining of the reporter have also not been successful to date. We are currently investigating the reasons for our inability to increase expression from the reporter and the reasons for nonresponsiveness after behavioral training.

We have begun a parallel experiment in mouse. Recently, it was shown that a simple 6x wild-type CRE transgenic mouse can report meaningful responses to physiological stimulation in hippocampal slices (LTP vs. L-LTP), and pharmacological intervention in dissociated neuronal cells. We have made similar lines of mice that contain reiterated CRE sites but have incorporated the use of vertebrate "insulators" to help alleviate position-dependent effects on expression. We are using fragments from the chicken globin locus control region, which has been shown in transgenic settings to have "insulator" function. Insulation is generally defined as position-independent, copy-number-dependent expression. These mice are currently being characterized, in anticipation of testing behavioral, physiological, and pharmacological stimulations. The mouse system has the distinct advantage that numerous behaviors are available for testing, many of which have known anatomical requirements for memory formation based on ablation studies. Part of our strategy is to select particular behaviors whose required anatomical regions show low basal expression in our reporter mice, thus increasing the chances of detecting new, training-induced staining.

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**Molecular and Genetic Characterization of dCREB2**

M. Belvin, B. Nyein, H. Zhou, J. Yin

One of the advantages of the fly system is the decreased amount of "molecular redundancy" among families of molecules. In mammals, the CREB, CREM, and ATF-1 genes have all been shown to be capable of responding to similar transduction systems to activate transcription from CRE-containing downstream genes, at least in cell culture systems. Another level of complexity is added by the numerous alternatively spliced forms which can be generated from the CREB and CREM genes. It is unclear whether this molecular richness is utilized in neurons, although it seems very likely given that analogous, alternatively spliced isoforms exist in both *Drosophila* and *Aplysia* neurons. Thus, a real molecular description of the events that occur on the dCREB2 gene in response to LTM formation is a challenge, although less daunting than the comparable description on the three mammalian genes.

We have begun to characterize the set of immunoresponsive protein bands detected in endogenous *Drosophila* head extracts on Western blots. Currently, we detect a group of bands in the 12–50-kD range that we suspect to represent endogenous protein forms. These proteins will need to be isolated and characterized biochemically before we can be sure of their identity. We have made new antibodies to try to sort out some of this complexity, although we have not been able to generate an activator-specific serum after a number of repeated efforts. Ultimately, we will need a spatial description of the tissue distribution of these forms.

The other obvious advantage of the fly system is the feasibility of doing forward genetics. We are very interested in isolating a (probable) lethal mutation in the dCREB2 gene, which will facilitate genetic, molecular, and behavioral analysis of the role of this gene in LTM formation and neuronal development. In addition, the availability of a lethal mutation will allow a strong screen for suppressors, which may, depending on the molecular nature of the mutation, reveal upstream and/or downstream interacting genes.

In parallel, we have continued our examination of the S162 mutation, a γ-ray-induced lethal complementation group which co-maps to the same X-chromosome cytological location as a dCREB2
probe. This mutation results in early larval lethality in homozygous females and hemizygous males. However, this lethality can be partially rescued by the dCREB2-b transgene, and almost full rescue is achieved when the transgene is induced. The interpretation of simple rescue experiments is complicated by the fact that background (presumed) genetic variability can also give partial rescue. This variability in partial rescue precludes the use of this mutation in a simple suppressor screen. Therefore, we are awaiting the first-level description of protein isoforms in wild-type flies before returning to a molecular description of the S162 mutation. Our current efforts to isolate a mutation in the dCREB2 gene involve developing an easy visible screen for CREB activity.

The initial attempt at an external, visible screen for CREB activity utilized the white gene. The protein is normally involved in the synthesis of the red eye pigment found in wild-type eyes. The white gene shows dosage sensitivity, with a linearity between the amount of protein and the eye color over a sixfold range. We created a series of insulated, 3x, 6x, and 9x CRE-white reporter flies where the white-coding region was placed under the control of a simple CREB-responsive promoter element, exactly like our LTM cell reporters (see above). However, none of these transgenic reporters were responsive to induction of the dCREB2-b transgenic blocker when they were crossed together. Therefore, we have moved to a luciferase-based reporter.

**CRE-Luciferase Transgenic Reporters**

M. Belvin, H. Zhou, J. Wallach, J. Yin

Luciferase offers a number of advantages as a reporter molecule, including reduced half-life as a protein, ease of measurement in crude extracts, ability to measure in a behaving fly, and sensitivity. We have made 3x wild-type CRE luciferase transgenic reporter flies and measured luciferase activity in crude total fly, total head, or total body extracts. These measurements, while easy to carry out, have a large amount of variability and therefore require multiple samples. However, when two independent transgenic lines are "entrained" on a strict 12-hour light:12-hour dark cycle, we find that the luciferase activity shows periodicity, with a major peak in luciferase activity at dusk. More significantly, when flies are entrained for 4-5 days and then put into a 24-hour darkness regimen ("free run"), the reporter shows the same periodicity, indicating that this CREB-responsive transcription is likely to be regulated through the circadian system. Examination of adult head sections with a luciferase antibody shows that the spatial staining pattern is similar, if not identical, to that of our β-galactosidase reporters, with heavy staining in the visual system (the eye, lamina, and medulla).

We are testing the feasibility of this system as an easy method to measure CREB activity, in anticipation of using it as a phenotype to score in a mutagenic screen. Our understanding of the biology that regulates the CRE-luciferase reporters might allow us to use this reporter system as a method to screen for mutations in aspects of the circadian clock, genes upstream of CREB in the circadian circuit, and in CREB itself.

**Neurotrophins in Drosophila**

M. Stebbins

The neurotrophins are neuronal growth-factor-related proteins that serve important functions in neuronal differentiation, survival, target-directed outgrowth, and cell-type determination. Recent data suggest very strongly that certain neurotrophic factors, especially brain-derived neurotrophic factor (BDNF), may also have significant roles in modulating synaptic function and plasticity-related growth processes. These molecules are conserved across mammalian species, and very recently, an invertebrate molecule was described. Our goal is to determine if there is a conserved family member in Drosophila and to utilize flies to (1) place the function of these molecules in the rich context of Drosophila neuronal development and axon guidance, (2) begin to understand the complex signaling pathways downstream from ligand binding, (3) utilize fly genetics to identify interacting genes, and (4) ask if they are involved in learning and LTM formation.

To begin this process, we are looking for evidence that conserved ligands or receptors exist in Drosophila. Two complementary experiments are being pursued. First, we have made inducible transgenic flies carrying truncated versions of the mammalian trkA and trkB receptors. The truncated trkB receptor is a naturally occurring splice variant that contains...
just the extracellular ligand-binding domain and very little of the intracellular signaling domain. Functionally, it is a dominant-negative receptor. The truncation in the trkA gene was made at approximately the same point as the shortened trkB isoform. These flies are currently being examined to see if induction of the receptor results in any neuronal developmental phenotype, which would suggest that there is an endogenous fly protein that can be titrated by ectopic overexpression of the mammalian receptor. These reagents also allow us to test the inducible effects of the transgene on learning and LTM formation.

To complement these experiments, the mammalian proteins (NGF, BDNF, and NT-3) are being injected into fly embryos, and the embryos are being examined for neuronal developmental defects. Phenotypes in this assay would suggest that there are fly receptors which are capable of recognizing mammalian ligands. Taken together with the transgenic experiments, we should be in a position to decide on which neurotrophin or receptor to concentrate and have functional information on approaches to cloning and identifying the gene(s). We, and others, initially tried to identify possible conserved genes using DNA-based low-stringency approaches. cDNA clones for trkA, trkB, trkC, NGF, BDNF, and NT-3 were used unsuccessfully to screen Drosophila genomic Southern blots at varying levels of stringency. It is expected that some type of functional assay may have to be employed to clone the trophic molecules or their receptors.

**Development of Spatial-Temporal Inducible Strategies**

M. Stebbin, H. Zhou, J. Yin

In *Drosophila*, we have begun adopting the tetracycline-inducible system for spatially and temporally regulated expression of genes. Transgenic flies are being made that contain both the forward and reverse systems, where the *trans*-activator is expressed ubiquitously, to test the practical aspects of temporal regulation. We are also putting the forward system under a mushroom body enhancer-promoter, which should delimit high-level expression of the *trans*-activator to the anatomical region that is required for learning. Finally, we are adopting the system so that the currently available enhancer-trap lines (which express gal4 in defined areas of the adult brain) can be used to drive expression of the *trans*-activator in these regions. By layering on the enhancer trap system to the tetracycline system, we should be able to achieve reasonable spatial and temporal regulation of expression with the large available collection of characterized enhancer lines.

In mice, there have been recent reports of successful use of the tetracycline system in the analysis of behavior. However, one of the limitations that currently exists is the slow kinetics of induction for the reverse system, since it relies upon withdrawal of tetracycline. Preliminary information on the forward system is that induction is faster, although it still requires a couple of days to achieve reasonable levels of the induced target gene product. We believe that one major contributing factor to the kinetic problem is that the *trans*-activator is expressed very poorly, partly due to a cryptic splicing event that results in nonfunctional RNA. We have removed this site, as well as rebuilding the gene using mammalian codon usage. Mice are currently being injected with various transgenes to test these improvements. The CaM kinase II promoter, whose high-level expression does not begin until 3 weeks after birth and is delimited to the forebrain, is being used to drive expression of the forward *trans*-activator. In addition, the globin LCR fragments are being used to insulate the target transgenes. If these fragments function as insulators, then almost all independent lines of the target transgene should be fairly equivalent in their response to the *trans*-activator. This property would greatly reduce the number of combinations of transgenes (*trans*-activator and target) that would be needed to test for any given gene which is being induced.

To facilitate analysis of *trans*-activator expression both in flies and in mice, we have initiated antibody production against the forward and reverse *trans*-activators. Current versions of the tetracycline system only use target reporter genes to measure *trans*-activator function, since *trans*-activator expression is very low. The only available data on *trans*-activator expression in transgenic mice uses RT-PCR to detect *trans*-activator RNA. We suspect that the cryptic (nonproductive) splicing of the *trans*-activator gene contributes to this problem.

**PUBLICATIONS**

Our research interest is to understand the neural basis of learning and memory. The approach undertaken is to analyze *Drosophila* learning and memory mutants. During last two decades, a number of *Drosophila* mutants have been isolated on the basis of their poor performance in a classic conditioning task. Some of these mutations affect learning, whereas others affect memory. Insights into the neural basis of learning and memory may be gained if we can understand how the functions of the nervous system are altered in these mutants. Our research has been focused on two aspects of the problem. First, we use these mutants to dissect signal transduction pathways that may be important for learning and memory. This study has provided unexpected insights into biochemical processes that may be important for learning and memory and has identified new components for further genetic manipulation of behavior. Second, we are developing preparations for electrophysiological and Ca++ imaging recordings of neuronal activity in the mushroom body of the live fly brain. This effort has allowed us to record, for the first time, neural activity in the central nervous system in response to odors in live flies, which provides a basis to examine neural coding of olfaction and ultimately to investigate the neural basis of olfactory-associated learning and memory in flies.

**Requirement of the NF1 Protein for Activation of Rut-Adenylyl Cyclase**

F. Guo, F. Hannan, Y. Zhong [in collaboration with T. Inger and A. Bernards, Massachusetts General Hospital]

The *rut* gene, isolated as a learning and memory mutant, encodes a Ca++/CaM-sensitive adenylyl cyclase (Rut-AC). Biochemical study by M.S. Livingstone has shown that Rut-AC is not involved in the cAMP synthesis activated by monoamine transmitters, such as dopamine, 5-HT, and octopamine. Our electrophysiological study has revealed that Rut-AC is involved in mediating neuropeptide transmission. The pituitary adenylyl-cyclase-activating polypeptide (PACAP38)-like neuropeptide activates both Rut-AC/cAMP/PKA and Ras/Raf pathways to modulate K+ currents synergistically. This result brought our attention to the Ras/Raf pathway for its potential role in learning and memory. To understand this pathway better, we examined the effects of those mutations that disrupt either activation or inactivation of Ras activity. Among them, PACAP38 response was eliminated by the mutations at the locus homologous to the human neurofibromatosis type 1 (*NF1*) gene (Fig. 1).

Mutations in the *NF1* gene lead to a common human genetic disorder that is identified by benign tumors of the peripheral nerves, hyperpigmentation, white matter lesions in the brain, learning disabilities, and many other manifestations. The NF1 protein, which contains a fragment similar to the GTPase activating protein for Ras (Ras-GAP), stimulates the intrinsic activity of Ras-GTPase and therefore inhibits biological activation of Ras. However, the NF1 protein may not act solely to regulate Ras; it may also function as an effector mediating signaling important for differentiation. The *Drosophila* homolog of NF1 is 60% identical to the human NF1 protein over its entire 2802-amino-acid length. Our study indicates that the defective PACAP38 response in *Drosophila* NF1 mutants could not be explained by failure to negatively regulate Ras activity. Instead, the defect is caused by the blockade of activation of *rut*-encoded adenylyl cyclase because the PACAP38 response could be fully restored by supplying drugs that stimulate the cAMP pathway prior to the perfusion of PACAP38 (Fig. 2).

The conclusion that NF1 is a crucial component for activation of the cAMP pathway is further supported by studying the developmental defect of NF1 mutants. The smaller body size of NF1 mutants caused by reduced cell size was not rescued by manipulating the Ras pathways, but by expressing additional catalytic subunits of cAMP-dependent protein kinase A (PKA). This finding leads to new insights into mechanisms of G-protein-mediated signal transduction and the pathogenesis, and possible the treatment, of human type-1 neurofibromatosis.
Molecular Basis of Learning Disability in NF1 Mutants
F. Guo

We are interested in examining learning and memory in Drosophila NF1 mutants. First, NF1 patients and NF1 mouse knock out display a learning disability. Second, activation of adenylyl cyclase, which is defective in the learning and memory mutant rut, is regulated by NF1. Thus, Drosophila NF1 mutants may serve as a model for understanding the molecular basis of NF1-related learning disabilities and as a model for the development of drug treatment for the NF1 learning disability.

Greatly facilitated by the help from Tim Tully’s lab here at the Laboratory, we have been able to obtain preliminary data showing that associative learning is defective (Fig. 3) in NF1 mutants, whereas the peripheral sensory modalities related to learning behavior are near normal. We hypothesize that this learning defect is caused by a blockade of activation of rut-encoded adenylyl cyclase, as indicated in the PACAP38 response, as well as other adenylyl cyclases. The rut mutants have been isolated as a learning and memory mutant. On the basis of this hypothesis, we are testing whether the learning defect

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**FIGURE 1** PACAP38-induced enhancement of K⁺ currents is abolished in NF1 mutants. The arrow indicates focal application of PACAP38 (5 µm). "Control" current traces were recorded before application of PACAP38. The time in seconds after pressure-ejection of PACAP38 is indicated at the top of the current traces.

**FIGURE 2** Restored PACAP38 response in NF1 mutants by forskolin. Forskolin (10–100 µM) was applied to mutant cells before perfusion of PACAP38.

**FIGURE 3** Learning defect in NF1 mutants. The performance index is in a scale where 1 indicates a perfect learning and 0 no learning. The learning score was obtained by using Tully-Quinn and procedure.

**FIGURE 4** Basal level of adenylyl cyclase activity in NF1 mutants. Adenylyl cyclase activity was assayed by using homogenized abdomen tissues of adult male flies.
observed in NF1 mutants can be rescued by expressing additional PKA catalytic subunits or by feeding flies with forskolin, a drug that stimulates adenylyl cyclase activity, bypassing the NF1 effect.

Ras-NF1 Complex in Activation of Adenylyl Cyclase

F. Hannan, L. Luo

The electrophysiological experiment presented earlier indicates that NF1 is crucial for activation of adenylyl cyclase, but it provides no clues as to how NF1 influences the activity of adenylyl cyclase. Because the yeast protein, IRA, is distantly related to NF1 and is involved in Ras-dependent activation of adenylyl cyclase, we are testing the idea that activity of adenylyl cyclase can be regulated by the Ras-NF1 complex. The cyclase activity was measured from homogenized Drosophila tissues, and it was shown that the basal level of cyclase activity was not affected in NF1 mutants (Fig. 4). We are examining how cyclase activity in wild-type flies and NF1 mutants is affected by additional Ras or mutant Ras. Although preliminary data were very interesting, the results were not consistent. We are now trying to find conditions that give rise consistency.

Recording and Imaging of Neuronal Activity of Mushroom Body

N. Wright

The mushroom body (mb) is a distinct central nervous system structure that is crucial for olfactory-related associative learning in insects, including flies. Electrophysiological recordings from mushroom bodies in other large insects, such as the bee, have provided rather exciting insights into the neural basis of learning and memory. To utilize learning and memory mutants of Drosophila for gaining further understanding, we must first overcome the technical difficulty of the small size of Drosophila brain and neurons, which has previously prevented any physiological studies of the fly central nervous system. We have developed several preparations. First, the activity of mushroom body neurons was recorded intracellularly from a dissected whole brain, in which mushroom body neurons were identified by expression of green fluorescence protein (GFP) in the GAL4 enhancer-trap lines. This preparation may allow us to study synaptic plasticity of mushroom body neurons. Second, the activity of mushroom body neurons was recorded from a live fly (Fig. 5). Third, to investigate the neural network properties that are related to learning and memory, we are developing a
preparation that allows us to monitor the activities of multiple mushroom body neurons by using Ca++ imaging. We have successfully obtained imaging of neuronal activity in response to odor stimulation.

**PUBLICATIONS**


In Press


**CELLULAR MECHANISMS OF ACTIVITY-DEPENDENT NEURONAL DEVELOPMENT**

H. Cline

N. Ali (URP)    J. Edwards    I. Rajan
R. Bari       I. Miloslavskaya     G.-Y. Wu
B. Burbach    E. Nedivi        D.-J. Zou

The lab is working toward an understanding of the cellular and molecular mechanisms that control the development of structure and function of the central nervous system (CNS). We perform our studies using the developing retinotectal projection of the frog *Xenopus*. We use several approaches to this system that allow us to assess and manipulate the coordinated development of neuronal morphology and synaptic transmission in the CNS. One approach is to use imaging of single retinal axons or tectal neurons in the intact anesthetized animal to observe their morphological development for up to 5 days in vivo with time-lapse confocal microscopy. A second approach is to use a whole-brain preparation to take whole-cell patch-clamp recordings from neurons at different stages in the process of maturation. We can record from tectal neurons as they receive their first synaptic contact from retinal afferents and track the development of the synaptic connections as they mature. This is one of the only experimental systems that permits direct recordings of synapse formation and maturation in the intact CNS. To manipulate the genetic and protein components of the neurons, we use viral vectors to deliver genes of interest into frog neurons. We are now using this range of techniques to test the hypothesized roles of synaptic activity and activity-regulated proteins in the coordinated development of the structure and function of the CNS.

**Dynamic Growth of Normal Axons**

H. Stier, I. Miloslavskaya, S. Witte, H. Cline

Changes in neuronal structure can contribute to the plasticity of neuronal connections in the developing and mature nervous system; however, the expectation that they would occur slowly precluded many from considering structural changes as a mechanism underlying synaptic plasticity that occurs over a period of minutes to hours. We have taken time-lapse confocal images of retinotectal axon arbors to determine the time course, magnitude, and distribution of changes in axon arbor structure within living *Xenopus* tadpoles. Images of axons were collected at intervals ranging from 3 minutes, 30 minutes, and 2 hours during total observation periods up to 8 hours. Branch additions and retractions in arbors imaged at 3- or 30-minute intervals were confined to shorter branches. Sites of additions and retractions were distributed throughout the arbor. The average lifetime of branches was about 10 minutes. Branches of up to 10 μm could be added to the arbor within a single 3-minute observation interval. Observations of arbors at 3-minute intervals showed rapid changes in the structure of branch tips, including transitions from lamellar growth cones to more streamlined tips, growth cone collapse, and re-
extension. Simple branch tips were motile and appeared to be capable of exploratory behavior when viewed in time-lapse movies. In arbors imaged at 2-hour intervals during a total of 8 hours, morphological changes included longer branches tens of microns in length. An average of 50% of the total branch length in the arbor was remodeled within 8 hours. The data indicate that the elaboration of the arbor occurs by the random addition of branches throughout the arbor, followed by the selective stabilization of a small fraction of the new branches and the retraction of the majority of branches. Stabilized branches can then elongate and support the addition of more branches. These data show that structural changes in presynaptic axons can occur very rapidly even in complex arbors and can therefore play a part in forms of neuronal plasticity that operate on a timescale of minutes.

Calcium Imaging in Retinal Arbors
J. Edwards, H. Cline

Now that we have documented the rates of morphological changes in retinal axon arbors and in growth cones, we have begun to test whether changes in $[\text{Ca}^{2+}]_i$ correlate with neuronal growth and growth cone navigation in vivo, and whether experimentally decreasing $[\text{Ca}^{2+}]_i$ changes these aspects of neuronal development. We have been able to image changes in $[\text{Ca}^{2+}]_i$ in the growing axon arbors and growth cones in vivo. Our initial experiments in this project indicate that branch additions are spatially and temporally correlated with transient increases in local calcium. More recent experiments have sought to determine the mechanisms that regulate intracellular calcium in retinal axon arbors. Continuing to work in the intact animal, we have found that light stimulus to the eye increases calcium in the axon terminals and that this increase is blocked by antagonists of the nicotinic acetylcholine receptor (nAChR) applied to the axon terminals in the optic tectum. Twenty years ago, it was reported that nAChR activity was required for the normal development of the topographic projection from the retina to the optic tectum, but no mechanism for this action has been found. Our data suggest that nAChR on retinal axon terminals can influence the topographic organization of the retinotectal projection through a calcium-mediated change in axon growth.

Development of Glutamatergic Retinotectal Synapses
G-Y. Wu, H. Cline [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

To examine the development of glutamatergic synaptic transmission in the vertebrate CNS, we developed a preparation of the isolated intact tadpole brain amenable to reliable single-fiber stimulation, whole-cell recording and dye labeling, pharmacological control of the extracellular environment, and transient expression with a recombinant viral vector. The tadpole optic tectum is particularly advantageous for the study of neural development because new neurons are constantly being generated in a proliferative zone in the caudal region of the optic tectum. Neurons from rostral tectum are chronologically older and morphologically more complex than neurons in caudal tectum, so that within a single preparation, there exist neurons over a range of developmental stages arranged along the rostro-caudal (RC) axis of the tectum.

Calcium entry through the N-methyl-D-aspartate (NMDA) receptor might trigger the stabilization of synaptic connections and neuronal morphology, by activating calcium-sensitive protein kinases, such as calcium/calmodulin-dependent protein kinase type II (CaMKII). CaMKII is a developmentally regulated protein kinase implicated in control of neuronal growth, synaptogenesis, and synaptic plasticity. CaMKII is activated by calcium entry through NMDA receptors and has several substrates relevant to neuronal development, including cytoskeletal proteins and the $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor. We tested the hypothesis that CaMKII activity in tectal neurons may be part of the signal transduction pathway that mediates activity-dependent changes in synaptic physiology and neuronal structure.

Whole-cell recordings from optic tectal neurons in Xenopus tadpoles were used to study the maturation of a glutamatergic synapse. The first glutamatergic transmission is mediated only with NMDA receptors and is silent at resting potentials. More mature synapses acquire transmission by AMPA receptors. This maturational program is mimicked by postsynaptic expression of constitutively active CaMKII. Newly formed synapses may be silent unless sufficient depolarization is provided by coincident activity which could activate postsynaptic CaMKII, leading to the appearance of AMPA responses.
Control of Neuronal Growth

G.-Y. Wu, D.-J. Zou, I. Rajan, I. Miloslavskaya, B. Burbach, R. Bari, H. Cline

Developing neurons must coordinate their morphological and functional development. If CaMKII activity were involved in the activity-dependent control of neuronal growth as well as synapse maturation, this might be a pathway by which neurons could coordinate various aspects of their development. In vivo time-lapse confocal images of retinal axons, taken by D.-J. Zou, have demonstrated that elevated tectal cell CaMKII activity results in retinal axons that have simpler morphologies due to a selective retraction of short branch tips. G.-Y. Wu tested whether CaMKII activity might also control the morphological maturation of tectal neurons using in vivo time-lapse imaging and immunohistochemistry. CaMKII is developmentally regulated in tectal neurons. Neurons in which CaMKII activity was elevated by viral expression of a constitutively active CaMKII (tCaMKII) have more stable dendritic structure than control neurons. Inhibition of CaMKII increased dendritic growth selectively in more nature neurons, which express endogenous CaMKII.

These observations on neuronal morphology, taken together with the electrophysiology data, in which it appears that elevated CaMKII promotes the maturation of synapses, suggest the following type of coordinated regulation of neuronal structure and function: When NMDA receptors are active and there is calcium influx, the resultant increase in CaMKII activity has two distinct effects on the tectal cell. One is to promote the maturation of the synaptic physiology and the other is to deter additional growth of the dendrite at the local site where CaMKII activity was elevated. As a result of the decreased branch addition and branch extension in the tectal neurons, the retinal axons retract the newly added short branch tips that have failed to establish synaptic contacts with target neurons. In our experiments, we increase CaMKII activity throughout the tectal cell, but in the normal neuron, NMDA receptor activity, calcium influx, and CaMKII activity would occur at discrete sites within the arbor corresponding to sites of converging inputs. This would result in highly localized control of synapse maturation and neuronal growth.

This model suggests that glutamate receptor activity will also influence tectal cell development. To test this hypothesis, I. Rajan took in vivo time-lapse images of single tectal neurons from normal animals and from animals treated with pharmacological blockers of the glutamate receptors, NMDA and AMPA receptors, as well as the sodium channel blocker tetrodotoxin. Her beautiful in vivo images from the treated and control animals will help determine the role of glutamatergic activity on neuronal development.

Development of New Recombinant Viruses to Test Protein Kinase Function In Vivo

D.-J. Zou, B. Burbach, H. Cline

To test the role of calcium-dependent signal transduction pathways involved in regulating the formation of the retinotectal projection, we plan to decrease the activity of several calcium-dependent endogenous enzymes in tectal neurons by expressing their specific inhibitory peptides. We began by determining the potency and specificity of peptide inhibitors on the activity of endogenous enzymes by doing kinase assays in tadpole brain homogenates. With these fundamental biochemical data in hand, we then went on to construct recombinant vaccinia viruses that encode a variety of peptides which specifically inhibit endogenous CaMKII or PKC. We have begun to test the effect of these reagents on the development of retinal axon and tectal cell morphology.

Candidate Plasticity Genes

E. Nedivi, I. Rajan, H. Cline, R. Bari [in collaboration with P. Worley, Johns Hopkins University]

We have begun to test the potential function of several activity-regulated genes, which were isolated through differential screening techniques by E. Nedivi in Y. Citri’s lab at the Weismann Institute and by P. Worley’s group at Johns Hopkins University. The candidate plasticity genes are regulated by neuronal activity and during development. Initial experiments indicate that expression of three candidate plasticity genes using recombinant vaccinia virus constructs results in three unique phenotypes with respect to neuronal development. These promising results indicate that the frog retinotectal system is an
excellent system in which to test the postulated roles of novel genes in activity-dependent neural development and synaptic plasticity in vivo.

Control of Cell Proliferation and Differentiation by NOS


N. Peunova and G. Enikolopov have shown that nitric oxide (NO) functions as an intercellular signal which triggers PC12 cells to switch from a proliferating state to a differentiated neuronal phenotype. We have begun to test whether NO might perform such a function in the developing optic tectum. Diaphorase staining, which labels cells that contain nitric oxide synthase (NOS), and NOS antibody immunostaining demonstrate that NOS-containing cells are located in the optic tectum just rostral to the caudomedial band of proliferating cells. We treated tadpoles with NOS inhibitors and labeled the population of proliferating cells by BrdU incorporation, followed by immunostaining for BrdU. NOS inhibitors appear to increase the number of BrdU-labeled cells in the optic tectum and to change the distribution of BrdU-incorporating cells. These results support the hypothesis that endogenous NO may serve to control cell proliferation in the developing brain in vivo.

PUBLICATIONS


THE BIOLOGY OF LEARNING AND MEMORY

A. Silva  P. Chen  K.P. Giese  R. Filipowski
O. Carvalho  J.H. Kogan  P.W. Frankland
N.B. Fedorov  V. Cestari  S. Kida
E. Friedman  J. Coblentz

Our laboratory is studying how the brain processes and stores information. Our strategy is to analyze the behavior and electrophysiology of mice with specific genetic modifications. Below is a summary of our research efforts during the last year.

BEHAVIORAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF MICE WITH A POINT MUTATION IN THE CALMODULIN-BINDING DOMAIN OF aCaMKII

At basal calcium levels, the unphosphorylated calcium calmodulin kinase II (CaMKII) is inactivated in less than 1 second, whereas autophosphorylated CaMKII might remain active indefinitely. Remarkably, after autophosphorylation at Thr-286, the affinity of the enzyme for Ca/CaM increases 1000-fold, from a $K_d$ of 15 nm to one of less than 20 pm, a change that could have a significant impact on calcium-dependent processes in cells. Previous biochemical studies showed that the substitution of Thr-286 by alanine in aCaMKII does not disturb enzyme function at high Ca/CaM but blocks its ability to trap Ca/CaM, and to remain active after Ca/CaM concentrations return to basal levels. Using the Cre/Lox system (see Fig. 1), we have generated this mutation in embryonic stem cells and have derived mutant mice. Like the
homozygous null mutants, mice homozygous for this point mutation have severe deficits in fear conditioning and in spatial learning (water maze). The water maze deficits resemble those that we observed in mice with ibotenic acid lesions of the hippocampus. For example, both the hippocampal lesioned mice and these point mutants can learn the visible-platform test of the water maze, suggesting that they have the required vision and motor coordination to perform this task, whereas being profoundly impaired in the spatial version of the task. Electrophysiological analysis showed that these mutants have normal synaptic transmission and normal augmentation but that long-term potentiation (LTP) is severely impaired at a number of frequencies and paradigms tested. Our results show that this LTP deficit is not due to either an increase in inhibition, a prepotentiation of untetanized synapses, or differences in release during the tetanus. These results suggest that the postsynaptic autophosphorylation of this kinase is important for LTP and also suggest a connection between the LTP deficit of the mutants and their spatial learning abnormalities. We are currently studying the mechanisms that underlie the LTP deficits of these mutants, as well as the impact of this deficit on the properties of place cells and on other forms of plasticity, such as that involving circadian rhythms and the development of neocortical circuits.

PRESYNAPTIC FUNCTION AND LEARNING AND MEMORY: STUDIES OF MICE WITH A HETEROZYGOUS MUTATION FOR βCaMKII AND MICE LACKING SYNAPTOPHYSIN

Studies in our laboratory have shown that mutations that affect certain forms of short-term plasticity without affecting LTP can disrupt learning and memory. To extend these studies, in the last year, we have analyzed mice mutant for two other presynaptic proteins (βCaMKII and synaptophysin). Mice heterozygous for the βCaMKII gene are healthy and devoid of obvious neurological abnormalities, such as seizures and ataxia. Studies with the spatial version of the Morris water maze using a variety of protocols did not detect any abnormalities in these mutants. Similarly, studies of cued and contextual fear conditioning also did not detect any deficits in these mutants. These results are in striking contrast with the behavioral phenotype of the αCaMKII heterozygote which show pronounced deficits in both the water maze and contextual conditioning tests. To determine whether βCaMKII affected synaptic plasticity, we have studied LTP and its reversal, paired-pulse facilitation, and posttetanic potentiation. These forms of plasticity also seem to be unaffected in these mutant mice. However, the mutants appear to show faster rates of synaptic depletion under continuous, prolonged stimulation. Interestingly, it does not seem that this increase in synaptic depletion compromises the animal's ability to learn and remember, thus demonstrating that not all deficits in synaptic function result in learning or memory deficits. The results described suggest that the role of this kinase in synaptic function is not critical for learning. Similarly, studies in our laboratory have also shown that mice devoid of synaptophysin perform normally in the Morris water maze, suggesting that the loss of this abundant presynaptic molecule (5% of protein in synaptic vesicles) does not affect hippocampal-dependent learning.

CREB AND MEMORY

CREB, the cAMP-responsive element-binding protein, is a transcription factor whose activity is modulated by increases in the intracellular levels of cAMP and calcium. Results from studies with Aplysia, Drosophila, and mice indicate that CREB-mediated transcription is required for long-term memory. Additionally, a recent study found that long-term memory for olfactory conditioning can be induced with a single trial in Drosophila expressing a transgenic CREB activator, whereas in normal flies with presumably lower CREB-mediated transcription levels, conditioning requires multiple spaced trials. This result suggests that CREB-mediated transcription is important in determining the type of training required for olfactory conditioning in Drosophila. Interestingly, studies with cultured neurons in Aplysia indicated that removing a CREB repressor promoted the formation of long-term facilitation, a cellular model of nonassociative memory. We have recently confirmed our previous results that mice lacking the α and Δ CREB proteins (CREBαΔ-) have abnormal long-term but not short-term memory tested in an ethologically meaningful task (food preference task). Importantly, spaced training can overcome the profound memory deficits of CREBαΔ- mutants. Increasing the intertrial interval from 1 to 60 minutes overcame the memory deficits of the CREBαΔ- mice in three distinct behavioral tasks: contextual fear conditioning, spatial learning, and socially transmitted food preferences. These results indicate that manipulations of CREB function
can affect the number of trials and the intertrial interval required for committing information to long-term memory. Remarkably, this effect of CREB function is not restricted to simple conditioning tasks—it also affects complex behaviors such as spatial memory and memory for socially transmitted food preferences.

**αCaMKII IS REQUIRED FOR THE STABILITY OF MEMORY**

The studies described above show that CREB is required for memory. Animals with lower levels of CREB learn but forget within hours. Many other studies in a number of species have identified a variety of manipulations that affect memory tested within minutes or hours after training. By comparison, little is known about molecular processes that specifically affect memory weeks and months after training. In the last year, we have shown that the αCaMKII heterozygous mutation disrupts spatial memory 3–10 days after training without having a measurable effect on memory tested 3 days after training. The delayed time course of this effect suggests that the memory instability is not due to a failure to synthesize new proteins immediately after training. Rather, some other consolidation process is compromised. For example, the proposed transfer of memories from hippocampus to neocortical areas may be disrupted by this mutation. Alternatively, with lower levels of this kinase, LTP or some other related process may become unstable over time.

Interestingly, our previous studies had indicated that the αCaMKII is also required for learning. These mutants require more training than normal mice to master the spatial version of the water maze. Nevertheless, after extended training, these mice master this task, and our results suggest that unstable memory is not simply due to weaker learning that fades away, since we obtained similar results with different training paradigms (overtraining). Current-
ly, we are looking at the stability of in vivo hippocampal LTP in these mutants.

ABNORMAL NEURONAL FIRING AND IMPAIRED LEARNING IN Kvβ1.1-DEFICIENT MICE

Most of our studies have focused on how synaptic plasticity affects learning and memory. However, neurons express other plastic properties that may not necessarily involve synaptic function but that could nevertheless be required for learning and memory. One example of this is K+ currents and their role in spike dynamics. Thus, we have initiated a research program designed to determine how changes in spike dynamics affect learning and memory.

The modulation of rapidly inactivating voltage-gated (A-type) K+ channels has previously been implicated in frequency-dependent spike broadening and in learning in invertebrates. To test for a similar function of A-type channels in mouse brain, we have deleted the K+ channel subunit Kvβ1.1, which confers A-type inactivation on mammalian Kv1 channels in vitro. In the last year, we have demonstrated that the loss of Kvβ1.1 in mice leads to a substantial decrease in the ratio of A-type versus delayed rectifier current in hippocampal CA1 pyramidal neurons. This change resulted in reduced frequency-dependent spike broadening. The amplitude of the Ca++-dependent slow after hyperpolarization (sAHP) was also reduced, being consistent with the idea that frequency-dependent spike broadening controls the Ca++ influx during a train of action potentials. The behavioral analysis of the Kvβ1.1-deficient mice revealed an impairment in reversal learning in the Morris water maze but not in the initial spatial learning. Additionally, 24-hour memory for socially transmitted food preferences is also abnormal in the mutants. These results indicate that Kvβ1.1 contributes to both A-type channel inactivation and frequency-dependent spike broadening regulating neuronal Ca++ influx. These are key neuronal determinants of spike dynamics, which may account for the behavioral deficits we have shown in the mutant mice.

IN VIVO ELECTROPHYSIOLOGICAL STUDIES OF HIPPOCAMPAL PLACE CELLS IN MUTANT MICE

At the heart of the work carried out in our laboratory is the connection between the behavioral and electrophysiological phenotypes of mutant mice. However, most of our electrophysiological studies have been restricted to the analysis of neurons in brain slices. Measurements in brain slices are the ideal way to study specific molecular and cellular mechanisms, such as synaptic and channel function. However, it is difficult to connect directly our electrophysiological studies with the behavioral phenotypes of the mutant mice because of the multisystem complexity of the brain. Changes in cellular function can be more directly connected with changes in circuit properties than with alterations in behavior. Similarly, the behavior of an animal may be more easily explained on the basis of circuit properties than with cellular biology. Therefore, the understanding of circuit properties may bridge cellular mechanisms with behavior.

One way to study the properties of circuits is to collect information about cell firing during behavior with techniques that allow simultaneous recording from multiple cells in a given circuit. For example, single-unit recordings in the hippocampus found that many cells seem to fire at higher frequencies when the animal is in a specific place (place cells), as if they were encoding a specific spatial location. Thus, multiple in vivo recordings of cell firing allow us to glimpse at the properties of cells in functioning neuronal ensembles.

Mutant mice are ideal tools to explore the connection between cellular physiology (LTP, LTP, SLP), circuit properties, and behavior. So far, single-unit recordings have only been used in a few mutant studies. The first were our collaborative studies with neocortical single-unit recordings which were used to study the impact of the loss of the αCaMKII on neocortical plasticity. In these studies, single-unit recordings were used to study responses to sensory stimulation before and after periods of deprivation. The results implicated this kinase in neocortical plasticity, both in the somatosensory cortex and in the visual cortex.

In the last year, Dr. Yoon Cho, previously in our laboratory, and Dr. Howard Eichenbaum developed electrophysiological methods for the study of place cells in mice. Yoon's work shows that it is possible to measure place cells in mice, a finding that has also been reported recently by two other groups. Her findings suggest that the relationship between place cells, LTP, and memory is likely to be complex, since a mutant lacking spatial learning and CA1 LTP nevertheless showed nearly normal place cells, suggesting that CA1 LTP is not essential for the establishment of place cells in the CA1 region.
THE INVOLVEMENT OF GRF IN LEARNING AND MEMORY

Previous studies in our laboratory showed that the mutation of the neurofibromatosis type 1 (NF1) gene in mice resulted in specific impairments in the Morris water maze: Spatial, but not visible, learning was affected. Fear conditioning was also unaffected in the NF1 mice. The NF1 gene product contains several domains, one of which has GAP activity that stimulates the inactivation of Ras. To test whether the mutation of another Ras regulator thought to function in the brain could also affect learning in a similar way, we generated mice with a targeted disruption of the exchange factor Ras-GRF. This Ras activator is expressed preferentially in central neurons, and it is activated by both calcium and cholinergic signals, two stimuli involved in learning. Similar to the NF1 mice, the Ras-GRF mutants showed impairments in spatial learning but not in fear conditioning. These results suggest that the deregulation of Ras can lead to learning impairments and that such deregulation may account for the learning deficits in NF1. Furthermore, the NF1 mutant mice may be a useful model to study how the mutation of the NF1 gene causes learning disabilities in humans.

SOCIALLY TRANSMITTED FOOD PREFERENCES: A NOVEL WAY TO TEST MEMORY IN MICE

Our laboratory is interested in hippocampal-dependent memory mechanisms. However, there are not many hippocampal-dependent tasks suitable to distinguish learning from memory deficits. Most hippocampal-dependent learning tasks, such as the water maze, involve multiple training sections, and therefore it is problematic to separate effects on learning and memory. Most of our memory studies have used fear conditioning tasks because the animal can learn with a single brief trial and the memory that is triggered is long lasting. However, fear conditioning involves foot shocking (aversive), and it does not reflect the natural behavioral repertoire of the mouse. Thus, in the last year, we have adapted the social transmission of food preferences test to mice. This task had previously been developed in rats. This behavioral test takes advantage of the fact that rodents develop a natural preference for foods that they have recently smelled on the breath of other rodents. It allows us to test whether mutants have memory deficits in a hippocampal-dependent test that involves neither aversive stimuli, spatial learning, nor fear conditioning. Importantly, this task exploits ethologically meaningful behaviors: the animals ability to learn quickly and remember information pertaining to social olfactory cues. The social transmission of food preferences test takes place in three phases (Fig. 2): First, "demonstrator" mice are given a distinctively scented food (ground mouse chow with cinnamon or cocoa). Second, the demonstrator mice are allowed to interact with "observer" mice, during which the observer mice have the opportunity to smell the scented food on the breath of the demonstrator mice. Third, at a given time later, the observer mice are given a choice between the food that the demonstrator mice had eaten or another novel food. Like rats, mice show a preference for the food eaten by other mice, and the memory for the food is stable through many days. Remarkably, an interaction as brief as 5 minutes is...
sufficient to trigger a memory that last many days. During the last year, we have used this task to study memory in a number of our mutants. With our help, many other laboratories are now using this task for the analysis of learning and memory in mutants.

**PUBLICATIONS**


**TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES**

R. Malinow

N. Dawkins  Z. Mainen  
J. Esteban  M. Maletic-Savatic  
Y. Hayashi  A. Shirke  
T. Kothan  S. Zaman  
H. Hinds (visiting MD-PhD Harvard/MIT student)  
C. Sano (visiting Japanese Medical Student)  
S. Horie (visiting Japanese Medical Student)

Our group addresses issues directed toward an understanding of learning and memory by studying the physiology of synapses. We study synaptic transmission in rat brain slices which are complex enough to show glimpses of emergent properties as well as simple enough to allow hard-nosed biophysical scrutiny. We have also begun to focus on neurons and synapses in cultured conditions. This allows us to study cell biological details with greater control. It is our philosophy that synapses have some key properties whose understanding is possible and that such an understanding will provide insight into phenomena at higher levels of complexity.

In Press


**Dendritic Exocytosis Is Mediated by Activation of CaMKII**

M. Maletic-Savatic

We continue to study a novel process we have discovered in neurons: calcium-evoked dendritic exocytosis (CEDE). This process is likely to be involved in the delivery of proteins to the surface membrane and may control the make-up of synapses. We have previously shown that CEDE is developmentally regulated and is mediated by the actions of CaMKII and have now found that intact microtubules are...
necessary for this process. We are currently investigating the identity of the organelles that undergo exocytosis by using combined light and electron microscopy.

What Is the Effect of CaMKII on Synaptic Transmission?

A. Shirke

We have previously shown that long-term potentiation, a form of synaptic plasticity, is mediated by the action of postsynaptic CaMKII. We have investigated the possible downstream effects of CaMKII by injecting an active form of CaMKII into neurons and determining the effects on glutamate receptor sensitivity to neurotransmitter. We find that injection of CaMKII into postsynaptic neurons increases synaptic transmission as well as sensitivity to exogenously applied neurotransmitter. Analysis of this increase suggests that this is due to an increase in the number of receptors, not an increase in the affinity of receptors. We will investigate further how CaMKII can increase the number of postsynaptic receptors. One possibility is that receptors are delivered to a synapse through a CEDE-like process.

LTP in Mice Lacking αCaMKII

H. Hinds

We have studied LTP in mice lacking the α isoform of CaMKII and find that LTP is diminished but not absent. Thus, genetic background appears to determine the requirement for αCaMKII. We find that LTP is exactly 50% of normal. Under various induction protocols, we obtain 50% of the normal LTP. These observations make predictions regarding which kinases are involved in LTP and at what stage in LTP generation.

How Is the Number of Glutamate Receptors in a Synapse Regulated?

Y. Hayashi, C. Sano, S. Horie

We have begun to investigate the cell biology of glutamate receptors; i.e., how are receptors delivered to the synapse, and what are the regulatory mechanisms involved? We are studying this using heterologous expression of fusion proteins in cultured neurons. We make recombinant glutamate receptors fused to green fluorescent protein (GFP) to monitor the mobility, association, and aggregation of synaptic receptors.

Silent Synapses

R. Malinow

The synapses we study (which are similar to and serve as a model for most excitatory synapses in the vertebrate brain) use glutamate as the neurotransmitter and have two types of postsynaptic glutamate-sensitive receptors: AMPA-type and NMDA-type receptors. These receptors differ in their activation properties as well as their permeation (what they allow to pass into the cell once activated). AMPA-type receptors need only glutamate to open, and NMDA-type receptors require both glutamate and membrane depolarization. Last year, we published direct evidence that a large fraction of the synapses we study have only NMDA-type receptors. These synapses will thus be silent (even if transmitter is released) when the postsynaptic cell is at resting membrane potential (most of the time), because the postsynaptic receptors will not open if only transmitter is released. We have reached this conclusion by looking at failure rates during transmission elicited when the postsynaptic cell is held at hyperpolarized and depolarized potentials. If there were AMPA-type receptors at all synapses, the failure rates should be the same; we find twice as many failures at hyperpolarized potentials. This difference is not present if NMDA-type receptors are blocked. This and several other pieces of evidence indicate that there are synapses with only NMDA receptors. We recently published several lines of evidence that pure NMDA synapses add AMPA receptors during LTP.

Developmental Progression of Silent Synapses

R. Malinow [in collaboration with G.Y. Wu and H. Cline, Cold Spring Harbor Laboratory]

We have looked at the developmental progression of these pure NMDA synapses in the retinotectal sys-
tem of the tadpole. We find that initial synapses are pure NMDA and silent at hyperpolarized potentials. During development, AMPA receptors are added. We also find that increased postsynaptic CaMKII can mimic this developmental maturation. These results have led to a specific hypothesis of synapse formation and modulation of gain by LTP: We find that initial synapses form with only NMDA receptors. These synapses will transmit information only if coactive with other synapses (which have AMPA receptors). If the pure NMDA-receptor synapses are potentiated by LTP, they will now have AMPA receptors added to them and will be able to transmit information without the need for coactivation with other synapses.

Appearance/Disappearance during the Estrous Cycle?

S. Zaman

During the estrous cycle of the rat (5d), there is a 30% increase and decrease in the number of synaptic contacts in the hippocampal CA1 region. We wish to test the hypothesis that these transient synapses have only NMDA receptor function. We are currently measuring AMPA- and NMDA-mediated responses in hippocampal slices from different stages in the estrous cycle. Using the methods previously established, we will be able to test directly if there are more pure NMDA synapses during the time when, anatomically, it is known that there are 30% increases in synapses.

Immunohistological Identification of Silent Synapses

J. Esteban, T. Koothan

We have generated polyclonal antibodies that recognize a putative extracellular domain of AMPA receptors. We are currently testing if this antibody can be used to detect synapses that have no AMPA receptors. Our preliminary results indicate that there are discrete regions in dendrites that are immunopositive only when the plaman membrane is permabilized, and not before permabilization. This is consistent with the existence of synapses that have AMPA receptors in intracellular regions and not on the surface.

We will use a similar strategy to determine (1) if there are indeed synapses (with presynaptic markers), (2) if these regions contain NMDA receptors, and (3) if the prevalence of such sites can be manipulated with LTP.

Is Synaptic Transmission in the Brain a Point to Point Process?

Z. Mainen

Although the traditional view of a synapse is that each presynaptic terminal releases transmitter that affects receptors on a single postsynaptic spine, there have been recent suggestions that this may not be so. It could be that transmitter released from one presynaptic terminal affects receptors on many nearby synapses. This is made plausible by the observation that some glutamate receptors (NMDA-type) have a 100-fold greater affinity for glutamate than other receptors (AMPA-type). Thus, any spillover of glutamate from one synapse could activate NMDA receptors on nearby synapses. This would have several important implications regarding information transfer and mechanisms of plasticity (e.g., since NMDA receptors need to be activated to induce LTP). Furthermore, this view would make us reinter-pret the findings regarding "silent synapses" described above.

We have examined some of the evidence that has been put forth by other groups supporting the spillover model. These studies have used extensively a putative indicator of presynaptic function: the coefficient of variation (CV) of synaptic responses. We find that this measure is inadequate when applied to NMDA responses. In particular, pure postsynaptic changes (like partial inhibition of NMDA responses with a high-affinity antagonist) produce robust changes in CV of the NMDA response. We show with experiments and detailed electrotonic models that this inadequacy of the CV is due to voltage-clamp errors. Therefore, conclusions based on CV of NMDA responses, used by a number of groups to argue for the spillover model, are nulled.
Cell Biology of Presenilin 1, a Protein Responsible for Familiar Alzheimer’s Disease
T. Koothan

A protein which when mutated is responsible for 50% of familial Alzheimer’s disease, PS-1, was recently cloned. Little is known regarding its cellular distribution and function. We have generated polyclonal antipeptide antibodies directed against the amino terminus (N) or postulated loop portion (L) of this protein. Using Western blot analysis, we find that this protein is cleaved in half and that this cleavage is developmentally regulated. We have evidence that this cleavage is controlled by calpain: Calpain inhibitors added to cells prevent cleavage and calpain added to cell lysates promotes cleavage. We also find that the PS-1 protein appears to form dimers. Using immunohistochemistry, we find that PS-1 is expressed only in neurons, not glial cells. The loop portion of the protein is localized in the lysosomes, whereas the N portion is localized in the endoplasmic reticulum. We plan to investigate the localization, processing, and function of this protein in neuronal function.

Electrophysiology of Transgenic Mice Carrying Mutated Presenilin 1
S. Zaman [in collaboration with S. Sisodia, A. Parent, D. Borcheit, Johns Hopkins University]

We have begun a collaboration with this group to study the electrophysiology of mice expressing a mutant form of PS-1 that is known to cause disease in humans. Preliminary studies indicate that various electrophysiological changes are aberrant in these mice. We will continue to characterize these changes and carry out experiments to determine the underlying mechanisms.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission. Such an understanding is necessary to derive a mechanistic flowchart of plastic processes. We also continue to probe the role of different molecules, including CaMKII and PS-1, in activity-induced and developmental synaptic plasticity.

PUBLICATIONS

SIGNAL TRANSDUCTION AND DIFFERENTIATION

G. Enikolopov  S. John  N. Peunova  Y. Stasiv
P. Krasnov  G. Ramanathan  A. Weissmann
B. Kuzin  V. Scheinker  O. Zatsepina

Our main interest lies in signals that link cell activity with cell differentiation. In the past year, we have concentrated most of our efforts on the role of nitric oxide (NO) in development. We are testing a hypothesis that NO acts as an antiproliferative agent and controls the balance between cell proliferation and cell differentiation during normal development. This project stems from our finding that NO is essential for neuronal differentiation of cultured cells. To see if this holds true for the intact developing...
organism, we started several projects with different models of animal development. We have shown that during Drosophila development, NO controls the cell number and, eventually, the tissue development and organ size of the developing fly. We are now focusing on genetic and biochemical interactions of NO with systems that control cell division and cell differentiation in the developing organism. Another major interest in the lab is protein trafficking in the neuronal cell. We want to determine motifs that are essential for trafficking of proteins to the terminals of neuronal cells, with a goal of targeting chimeric proteins to the nerve terminals.

**Nitric Oxide Regulates Cell Proliferation during Drosophila Development**

B. Kuzin, N. Peunova, V. Scheinker, G. Enikolopov [in collaboration with I. Roberts, Cambridge University]

Organ development requires a tightly controlled program of proliferation followed by growth arrest and differentiation and, often, programmed cell death. The balance between the number of cell divisions and the extent of subsequent programmed cell death in imaginal discs of Drosophila larvae determines the final size of organs and structures of the adult fly. We have found that NO acts as an antiproliferative factor and controls cell number during Drosophila development. Nitric oxide synthase (NOS) gradually accumulates in imaginal discs of Drosophila and reaches maximum levels at the end of the larval development, when DNA synthesis and cell division in most of the cells of imaginal discs and the brain slow down. Inhibition of NOS in larvae results in surplus cell proliferation and excessive growth of the segments of the body of the adult fly. In contrast, ectopic overexpression of the NOS gene causes a decrease in the number of cells and reduction in the size of some segments. The distribution of affected segments in the adult leg corresponds to the distribution of NOS in the larvae, and the changes in segment size can be directly correlated with changes in DNA synthesis in imaginal discs after manipulations of NOS activity. The results of BrdU labeling of imaginal discs suggest that modulation of NOS activity directly affects the number, but not the distribution, of cells in S phase. However, in some imaginal discs (e.g., the eye disk), we consistently detected an increase in the number of cells in S phase after inhibition of NOS, but the resulting adult eye usually appeared to be normal. We studied the possibility that programmed cell death counteracts excessive cell proliferation and restores the normal number of cells in the eye during metamorphosis. We tested whether the potential surplus cell proliferation can be unmasked in the absence of programmed cell death. We used transgenic flies in which programmed cell death in the developing eye was suppressed by recombinant p35, an inhibitor of apoptosis, to reveal excessive proliferation after NOS inhibition. We found that under these circumstances, several cell types and structures are overrepresented, the most noticeable change being an overall increase of the size of the eye due to the increased number of ommatidia. In addition, other cell types (e.g., secondary and tertiary pigment cells, cone cells, and cells of the bristles) proliferated after NOS inhibition to levels higher that those achieved by blocking apoptosis by p35. These data demonstrate that the removal of the suppressive influence of NO leads to an increased size of the adult organ, unless this effect is masked by programmed cell death, and indicate that the final cell number in the adult organ is under dual control of both cell proliferation and programmed cell death. Furthermore, these data provide independent support for the hypothesis that NO directly regulates the cell number during development.

We propose that production of NO is required during embryonic development and during tissue regeneration in the adult organism for the proper control of cell proliferation. The antiproliferative properties of NO may be particularly important in situations in which terminal differentiation of committed cells is temporally separated from cell proliferation and is strictly dependent on cessation of cell division. Given the multiplicity of the NOS isoforms, their overlapping tissue distribution, and the possibility that NO can be transferred within the developing organism by hemoglobin and thus supplied exogenously by the mother, it is conceivable that any group of cells in the embryo and fetus can be exposed to NO action.

Our current efforts are focused on dissecting genetic interactions of NO during Drosophila development. We use mutant and transgenic flies carrying components of the retinoblastoma pathway to study cross talk between the NO pathway and the general cell cycle machinery in controlling the final size of organs of the adult fly.
Molecular Mechanisms of Nitric Oxide Synthesis during Drosophila Development

Y. Stasiv, G. Ramanathan, B. Kuzin, G. Enikolopov [in collaboration with M. Regulski, Cold Spring Harbor Laboratory]

It has been recently shown in our lab that manipulation of NOS activity in Drosophila larvae results in dramatic changes in the adult fly’s organs (Kuzin et al. 1996). This argues that NO controls the size of body structures during Drosophila development. Recently, a Drosophila NOS gene, dNOS1, was cloned (Regulski and Tully, Proc. Natl. Acad. Sci. 92: 9072 [1995]) that is active in adult Drosophila brain and whose closest analog is mammalian neuronal NOS. We sought to identify Drosophila genes that are responsible for NO action in development. We used several approaches to clone genomic or cDNA copies of NOS-related loci. Our results suggest that, in contrast to mammals, where NO is synthesized by three different NOS genes, Drosophila most probably has only one locus for NOS, which is the analog of mammalian neuronal NOS. However, different dNOS transcripts are expressed during development. We have cloned one of them, dNOS3. dNOS3 is expressed mainly in Drosophila larvae. It arises as a result of alternative splicing which results in early termination of translation and synthesis of a 214-amino-acid-long protein, which corresponds to the amino-terminal part of the dNOS1 protein. Currently, we are testing the possible functional role of dNOS3 by coexpression with dNOS1 in mammalian and Drosophila cell lines and by generation of transgenic lines expressing dNOS3 under the control of inducible (heat shock) and tissue-specific promoters.

NO and Xenopus Brain Development

N. Peunova, G. Enikolopov [in collaboration with H. Cline, Cold Spring Harbor Laboratory]

Our studies reveal that synthesis of NO is essential for the balance between proliferation and differentiation during larval development, thus contributing to the final size of organs of the adult fly. We are interested in how conserved this mechanism of growth control is and whether it is similarly involved in vertebrate morphogenesis. We chose Xenopus laevis as a model organism for these studies, focusing our investigation on the formation of the brain. Histogenesis of the Xenopus brain begins with the folding of the neural tube and continues until metamorphosis. New cells are continuously produced in the ventricular zone of the tadpole brain, and they undergo terminal differentiation while moving outward of the ventricle. Using cytochemical and immunochemical staining, we have found that NOS is accumulated in maturing neurons, suggesting that it may be involved in cessation of proliferation of neural precursors. To test whether NO has a causative role in establishing growth arrest, we are trying to perturb NOS expression by introducing a slow-releasing plastic impregnated with NOS inhibitors in the ventricle of the tadpole brain. Our initial results suggest that NOS is directly involved in control of cell proliferation and neuronal differentiation in the developing vertebrate brain.

NO and Neuronal Differentiation

N. Peunova, O. Zatsepina, V. Scheinker

Our studies indicate that NO has a dual role during nerve growth factor (NGF)-induced neuronal differentiation of PC12 cells: First it acts as an antiproliferative agent, inducing growth arrest in dividing cells, and thereafter, when growth arrest is firmly established and the cells have undergone differentiation, it helps suppress programmed cell death. Inhibition of NOS at early stages of NGF action, in yet undifferentiated cells, results in continuous cell division and suppression of differentiation, whereas in fully differentiated PC12 cells, it leads to apoptosis. We study mechanisms of this dual mode of action of NO. To examine the contribution of different NOS isoforms in these two phases of differentiation, we followed the expression of various NOS genes by Western blot analysis and by reverse transcriptase–polymerase chain reaction (RT-PCR). Our results suggest that differentiation of PC12 cells is accompanied by a switch in expression of different NOS isoforms. In particular, expression of the neuronal isoform reaches maximal levels after growth arrest was established and, perhaps, is more directly associated with survival functions.
Protein Targeting to Neuronal Cell Terminals
P. Krasnov, G. Enikolopov

The main aim of this project is to define the minimal protein motifs sufficient for protein trafficking to the terminals of neuronal cells. We want to use these motifs in protein vectors to target chimeric proteins capable of modulating signal transduction cascades to the neuronal terminals. This may enable manipulation of signaling events at the terminals without affecting the rest of the cellular compartments. We have chosen synaptotagmins as a starting material for a targeting protein vector. Synaptotagmins belong to a large family of evolutionary conserved proteins expressed predominantly in neuronal cells. Synaptotagmins are constituent components of synaptic vesicles and large dense-core secretory vesicles, and some synaptotagmin isoforms are highly enriched in neuronal cell terminals.

To define the structural elements of the synaptotagmin molecule that are responsible for its targeting properties, we performed extensive deletion and alanine scanning mutagenesis of rat synaptotagmin II tagged with an influenza virus hemagglutinin (HA) epitope. The mutants were transiently expressed in NGF-differentiated PC12 cells and their intracellular distribution was monitored by immunofluorescence and subcellular fractionation using anti-HA antibodies. We found that there are two structural elements of the synaptotagmin gene located near the transmembrane domain and in the carboxy-terminal domain, which are both necessary and sufficient for targeting of the protein molecule to the terminal. Mutations in the transmembrane region of synaptotagmin abolishes its incorporation in the vesicles, whereas deletions in the carboxy-terminal domain disrupt its proper accumulation in the terminals of neurites. This suggests that after the protein is incorporated in correct membrane compartments via its amino-end region, the transport machinery utilizes the signals at the carboxy-end of the molecule for proper transport, retention, and eventual accumulation at the terminal. Using alanine-scanning mutagenesis of the carboxy-terminal domain, we have identified two amino acids that are absolutely critical for accumulation of synaptotagmin at the terminal. These amino acids are conserved among all known synaptotagmin isoforms, suggesting that different synaptotagmins may have similar or common mechanisms of targeting. We are currently using the delineated sequences to target exogenous proteins involved in signal transduction to the terminals of neuronal cells and to search for interacting proteins which might participate in synaptotagmin’s trafficking in the neuronal cell.

We greatly appreciate the expert assistance of Santosh John in all of the described experiments.

PUBLICATIONS


In Press

In 1986, Cold Spring Harbor Laboratory began a Junior Fellow program to encourage independent research by outstanding young scientists who, during their graduate studies, displayed exceptional promise of becoming leading scientists of the future. The purpose of this program is to provide an opportunity for these young scientists to work independently at the Laboratory for a period of up to 3 years on projects of their choice. Junior Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free from other distractions. The interaction among research groups at the Laboratory and the program of courses and meetings on diverse topics in molecular biology contribute to a research environment that is ideal for innovative science by these Fellows.

Two previous Cold Spring Harbor Fellows, Dr. Adrian Krainer (1987) and Dr. Carol Greider (1988), are currently members of the faculty at the Laboratory. Dr. David Barford, our Junior Fellow since 1991, left in 1994 to go to Oxford University (Laboratory of Molecular Biophysics) as a university lecturer. Dr. Ueli Grossniklaus joined us in 1994 from the Department of Cell Biology, University of Basel, Switzerland. Dr. Scott W. Lowe, came from the Center for Cancer Research at the Massachusetts Institute of Technology. Both Drs. Grossniklaus and Lowe were promoted to the faculty as Assistant Investigators during this year so that they could expand their laboratories.

DEVELOPMENTAL GENETICS OF PLANT REPRODUCTION

U. Grossniklaus  R. Baskar  M. Hoeppner
N. Castiaux  J. Moore
W. Gagliano  J.-P. Vielle Calzada

Our research focuses on the characterization of the molecular and genetic basis of plant reproduction, in particular the formation of the female gametes. Unlike in animals, where the meiotic products differentiate directly into gametes, the spores of plants give rise to multicellular haploid gametophytes that produce the gametes later in their development. In flowering plants, the sexually dimorphic gametophytes (micro- and megagametophytes) develop within the reproductive organs of the flower. A single cell within an ovule primordium differentiates into a megaspore mother cell (MMC), undergoes meiosis, and produces four megaspores, three of which die. The fourth divides through three consecutive mitoses, to form the embryo sac consisting of seven cells: the egg cell, two synergids, three antipodals, and a binucleate central cell. After double fertilization of both the egg and the central cell, the ovule develops into a seed. Although the seven cells of the megagametophyte are of clonal origin, they develop along four alternative developmental pathways. The molecular and genetic basis controlling female gametogenesis in plants are largely unknown. The highly polar nature of the female gametophyte, its small number of distinct cell types, and its closely coordinated development with surrounding tissues of the ovule make it an ideal system to study fundamental aspects of plant development. The goal of our research is to investigate the role of positional information, cell lineage and cell-cell communication in plant morphogenesis, and cellular differentiation. A better understanding of the molecular mechanisms controlling megagametogenesis will not only yield important insights into fundamental concepts in plant development, but also provide tools for the manipulation of the reproductive system. We are particularly interested in applying our findings to the engineering of apomixis, an asexual form of reproduction through seeds. The introduction of apomixis into sexual crops would have revolutionary implications for plant
breeding and agriculture, allowing for the immediate fixation of any desired genotype and its indefinite clonal propagation.

We would like to acknowledge the efforts of a number of high school and undergraduate students who assisted in our research. Hillel Sims, John F. Kennedy High School, Bellmore, identified Arabidopsis homologs of genes required for meiosis in yeast; Paul Pultorak, SUNY Stony Brook, put some of our insertional mutants onto the genetic map; and Bill Wagner, Duke University, helped with the characterization of mutants disrupting megagametogenesis.

Genetic Analysis of Plant Reproduction by Enhancer Detection


To dissect female gametogenesis in plants, we are performing a large-scale enhancer detection/gene trap screen in Arabidopsis thaliana, a small crucifer that has been widely adopted as a model system for plant developmental biology and genetics. Enhancer detection and gene trap systems allow the identification of genes based on their pattern of expression. The isolation of genes expressed during megasporogenesis and megagametogenesis will yield important insights into the genetic control of plant reproduction. We are using an enhancer/gene trap system based on the Ac/Ds transposon of maize which was developed here at the laboratory (Sundaresan et al., Genes Dev. 9: 1810 [1997]). The presence of a transposon at a locus of interest greatly facilitates its subsequent molecular and genetic characterization. During the past 3 years, we generated about 4300 lines carrying single randomly distributed enhancer detector/gene trap transposons (transposants) by screening the progeny of close to 22,000 plants for unlinked transposition events. We have screened approximately 1500 of our transposants for reporter gene expression in developing ovules and 5000 transposants for mutations that disrupt female gametogenesis. The characterization of transposants at various stages of reproductive development is an ongoing long-term project in our laboratory.

Molecular Characterization of Insertions by TAIL PCR

To obtain molecular information on transposants that were identified in our initial screens, we isolated genomic fragments flanking the insertion site. We adapted a novel polymerase chain reaction (PCR)-based method, thermal asymmetric interlaced PCR (TAIL PCR; Liu et al., Plant J. 8: 457 [1995]), to isolate genomic regions flanking the Ds insertions. We designed three nested Ds primers each to isolate both 5'- and 3'-flanking regions. They are used in combination with an arbitrary degenerate primer hybridizing to many locations in the genome. Using unique thermal cycling conditions and consecutive rounds of PCR, it is possible to amplify specifically the genomic fragment flanking the insertion site. We have isolated flanking genomic regions for 87 transposants (93% success rate for the recovery of at least one border fragment) using two different arbitrary primers. Approximately 170 PCR products representing 87 loci that show reporter gene expression in developing ovules or disrupt megagametogenesis were directly sequenced. We identified 27 insertions into or nearby known genes or expressed sequence tags (ESTs) from Arabidopsis, 8 sequences with significant homology and 12 sequences with weak homology with other sequences in the databases. Whereas many of the sequences showed homology with ESTs of unknown function, others were similar to genes encoding basic cellular factors involved in metabolism, general transcription, and translation. Importantly, we also identified genes encoding putative regulatory proteins involved in signal transduction processes and transcriptional regulation which may serve key regulatory functions in sexual reproduction.

Developmental Alternatives during Megasporogenesis

J.-P. Vielle Calzada, U. Grossniklaus

The alternation of generations is one of the major developmental changes in the life cycle of a flowering plant. It involves the transition from a massive diploid plant body into a structure composed of a small number of highly organized haploid cells, the gametophytes. This transition is initiated with the differentiation of the MMC, followed by meiosis and completed with the differentiation of the functional
megaspore. There are numerous examples of developmental alternatives that lead to the formation of unreduced gametes, one of the key components of apomictic reproduction. The developmental versatility of megasporogenesis in flowering plants suggests a flexible regulatory control, the genetic and molecular basis of which is poorly understood. Only a few mutations disrupting megasporogenesis have been described, and genes functionally involved in megaspore specification and the initiation of female gametophyte development have yet to be isolated and characterized. Genetic analysis suggests that the differences between sexual and aposporous apomictic reproduction may be determined by a regulatory locus that directs somatic cells to form a female gametophyte without undergoing meiosis. It has been proposed that genes controlling apomixis may encode proteins which normally function during megasporogenesis in sexual reproduction, but may have an altered temporal and spatial pattern of expression in apomictic ovules. A better understanding of the molecular mechanisms underlying megasporogenesis is required to induce developmental deviations that resemble apomictic processes. To identify and clone genes acting during megasporogenesis, we screen our transposant collection for reporter gene expression in early stages of ovule development. In a pilot screen, we identified several lines that are characterized by reporter gene expression in ovule primordia prior to MMC differentiation, in megaspores undergoing meiosis, or in highly restricted regions of the developing ovule. Of particular interest is a semisterile line with polar expression restricted to proximal regions of the nucellus, a domain implicated in megaspore specification. The gametophytic control of this phenotype indicates a requirement for the haploid genome in late events of megasporogenesis. To confirm patterns of gene expression, we are currently establishing a high-resolution whole-mount in situ hybridization procedure that will allow us to detect signals at single-cell resolution in the ovule.

Dissection of Megagametophyte Development by Insertional Mutagenesis

J. Moore, W. Gagliano, U. Grossniklaus

Very few mutants affecting the gametophytic phase of the plant life cycle have been described in the literature, and the isolation and characterization of additional mutants will provide new insights into the genetic regulation of this process. In more than 5000 transposants, we identified 178 mutants with reduced fertility, suggesting a possible defect in megagametophyte development or function. If a mutation affects megagametogenesis, ovules carrying a defective female gametophyte arrest and do not develop into seeds. Therefore, a plant heterozygous for an insertion affecting megagametophyte development is expected to display a semisterile phenotype, since half of the ovules will contain a gametophyte carrying the mutant allele. Reduced fertility can result from poor growth conditions, sporophytic female sterile mutations with low penetrance, reciprocal translocations, or mutations affecting the female gametophyte. These different possibilities can be distinguished by a segregation analysis of the kanamycin resistance marker present on the Ds element. Insertional mutations that disrupt megagametogenesis will show a non-Mendelian segregation of the Ds element. We characterized the segregation pattern of the kanamycin resistance gene in 126 lines and identified 29 transposants with distorted segregation consistent with a gametophytic defect and 30 additional lines where gametophyte transmission was affected but masked by a second element. These results suggest that approximately half of the initially identified mutants are closely linked to a Ds insertion (and therefore likely to be tagged) and that about 1% of all insertions affect gametophytic transmission.

We chose 19 lines carrying a single element and displaying a consistent phenotype for further analysis. The morphology of affected megagametophytes was characterized in cleared specimens (Fig. 1). The different mutants disrupted various aspects of megagametophyte development such as free nuclear divisions, nuclear migration and differentiation, cellularization, and fertilization. We isolated and sequenced genomic regions flanking the insertion site for all 19 transposants, and 13 of them showed similarity to sequences deposited in the databases. Three major classes of genes that are expected to result in gametophyte lethality were identified: (1) insertions in essential genes with basic metabolic functions, for example, diTP-glucose-4-6-dehydratase or dihydrofolate reductase; (2) mutations in cell cycle control genes, for example, a disruption of a subunit of the origin recognition complex, Orc2; and (3) insertions in genes with a putative regulatory function controlling megagametogenesis and fertilization such as insertions in genes that have homology with
proteases, phosphatases, and other signal transduction molecules.

**Cell Specification in the Female Gametophyte**

R. Baskar, J. Moore, W. Gagliano, U. Grossniklaus

The small number of clonally derived cell types in the female gametophyte makes it an ideal system to study cell specification processes in plants. By characterizing genes that are specifically expressed in individual cells of the megagametophyte, we try to investigate the respective roles of positional information, cell lineage, and cell-cell communication in cell determination and differentiation. We have screened close to 1500 transposants for reporter gene expression in mature ovules. Among the enhancer detector transposants, approximately 5% show expression in the female gametophyte. Whereas some of these show expression in all cells of the megagametophyte, others are specific to a subset of cells (e.g., the three cells of the egg apparatus) or to individual cell types such as the oocyte, synergids, and antipodals, suggesting that they may be involved in cell specification processes. We are particularly interested in the specification and function of the egg cell. To date, we have identified two transposants with expression in the egg, neither of which displays an obvious mutant phenotype. One of them carries an insertion in the carboxy-terminal region of a cdc2-like kinase; the second does not have significant similarity to other proteins in the databases. We are currently isolating
the promoter regions of these genes and will use them for genetic cell ablation and the misexpression of regulatory genes. Such experiments will yield important insights into the mechanisms of cell specification and allow us to probe the potential of the egg cell for autonomous activation, an important component of apomictic reproduction.

**Polyspermic Fertilization Events in Maize**

U. Grossniklaus, N. Castiaux

In flowering plants, two pairs of cells participate in the fertilization process. One of the two sperm cells delivered by the male gametophyte fuses with the egg to form the zygote, whereas the second fuses with the central cell and gives rise to the primary endosperm. Due to the inaccessibility of plant gametes, the fertilization process itself has not been studied extensively. Most animal species studied prevent the union of more than two haploid cells and have evolved systems to prevent polyspermy. In many animal species, a transient fast block to polyspermy is established by the depolarization of the egg membrane within milliseconds after the binding of the first sperm, and a second more permanent block is then generated by enzymatic changes in the egg membrane and the surrounding extracellular matrix. Although in vitro fertilization experiments suggest that the plant egg completes its cell wall within minutes after fertilization (Kranz *et al.*, *Plant J.* 8: 9 [1995]) thereby preventing further fertilization events, it is not known whether plant gametes prevent polyspermy by a fast block. We tested whether multiple sperms can participate in the fertilization of the central cell in maize. The central cell is binucleate and forms the triploid endosperm after fertilization. Normal endosperm development strictly requires the presence of maternal and paternal genomes in a ratio of 2:1, any deviation from this ratio resulting in kernel abortion. We crossed tetraploid females with a mixture of pollen from two different diploid males carrying distinguishable endosperm markers. In this cross, only kernels with endosperm containing two paternal genomes will fully develop. This is achieved if two sperm cells participate in the fertilization event with the central cell or if an aberrant meiosis produces diploid sperms. In 175 crosses, we recovered 26 full-sized kernels carrying the endosperm markers from both male parents, and 166 carried one of the two markers (Fig. 2). Since we only detect half of the polyspermic events in such a cross, about one quarter of the full-sized kernels (52/192) are derived from events where two sperm cells participated in the fertilization of the central cell. These results clearly demonstrate that polyspermy occurs in vivo and may explain the relative tolerance of plants to polyploidization and hybridization.

**Functional Analysis of medea, a Gametophytic Maternal Effect Mutant**

U. Grossniklaus, J.-P. Vielle Calzada, M. Hoeppner, W. Gagliano

We continued our characterization of medea (*mea*), a gametophytically controlled mutant that affects the subsequent sporophytic generation. We had shown that *mea* is a gametophytic maternal effect embry-lethal mutant: Seeds derived from *mea* eggs abort irrespective of the paternal contribution and gene dosage. Our genetic characterization of *mea* suggested that it affects either a maternally expressed cytoplasmic factor or an imprinted gene expressed from the maternal allele. To better characterize the *mea* phenotype, we compared the morphology of *mea* and wild-type seeds in cleared and sectioned specimens. Early stages of *mea* embryogenesis were indistinguishable from wild type, with the first differences becoming apparent at the globular stage. Although morphogenesis in wild-type embryos proceeds to the heart stage, *mea* embryos remain

![Figure 2](image_url)

**Figure 2** Full-sized kernels derived from a cross between a tetraploid female and a mixture of pollen from two diploid males. (a) Kernel carrying two paternal copies of the R-nj marker pigmenting the crown; (b) kernel carrying two paternal copies of R-st yielding a spotted pattern; (c) kernel derived from a polyspermic fertilization event carrying both the R-nj and R-st markers originating from two different paternal plants.
globular for an extended period of time undergoing additional cell divisions. By the time wild-type siblings have reached the cotyledonary stage and seed maturation begins, mea embryos have proceeded to the late heart stage but are much larger than wild-type embryos of the corresponding stage: mea late heart stage embryos have almost twice as many cell layers (19.6 +/- 1.1) as compared to wild type (13.0 +/- 0.9). Despite this dramatic increase in size, morphogenesis of mea embryos proceeds normally but is delayed, producing giant heart stage embryos that eventually die during seed desiccation. The development of the endosperm is also affected, showing a reduction of the rate at which nuclear division takes place but normal morphogenetic progression. Taken together, these results suggest that MEA regulates growth during embryogenesis and seed development without interfering with morphogenesis, which proceeds normally but slow.

To determine the function of the MEA wild-type product, we investigated whether mea is a gain-of-function or loss-of-function mutation. Since dominance and recessiveness are defined as an interaction of two alleles, this question cannot be addressed in haploid gametophytes. We constructed tetraploid plants carrying a mutant mea allele which allowed us to analyze seeds derived from diploid megagametophytes carrying two, one, or no mutant mea allele. This analysis showed that mea behaves as a recessive mutation and thus that the wild-type function of MEA is to restrict proliferation during embryogenesis. Our findings provide strong support for a theory proposed by Haig and Westoby (Am. Nat. 134: 147 [1989]) which explains parent-of-origin-dependent regulation of embryo size as the outcome of a conflict between parental genomes over the allocation of nutrients from the mother to its offspring. The theory predicts that parentally controlled loci (imprinted genes or cytoplasmic factors produced prior to fertilization) should influence the growth rate of the embryo, with paternally expressed genes promoting growth and maternally expressed genes tending to reduce it. mea is the first single gene mutation in plants providing strong support for this theory: The mea phenotype is under strict gametophytic maternal control, and mea embryos are characterized by increased proliferation and delayed differentiation. We have isolated genomic regions flanking the mea insertion and have recently identified a rare cDNA in a floral library. We are currently characterizing the structure and function of mea at the molecular level.

Arabidopsis Mutants Displaying Apomictic Traits

U. Grossniklaus, R. Baskar [in collaboration with R. Pruitt and S. Lolle, Harvard University]

Seeds are generally considered to be the result of sexual reproduction. In more than 400 apomictic species, however, seeds form independent of fertilization. Gametophytic apomixis can be interpreted as a short-circuiting of the sexual pathway where meiosis is impared or absent, and an unreduced cell produces a mature female gametophyte. The apomictic egg cell develops autonomously to produce clonal seeds. We have previously established a powerful screen to identify Arabidopsis mutants that allow seed development in the absence of fertilization. Such mutants display certain aspects of apomictic reproduction, and their identification and characterization constitute an important step toward the engineering of apomixis in sexual species. Taking advantage of a temperature-sensitive male sterile mutation isolated in R. Pruitt's laboratory, we performed a saturation screen for mutants that allow seed pod development under restrictive conditions. Among the more than 15,000 mutagenized plants, we identified close to 500 putative mutants which are currently being rescreened at Harvard. We expect to recover several distinct phenotypic classes: (1) suppressors of male sterility allowing the production of functional pollen, (2) revertants of the male sterile mutation, and (3) pseudo-suppressors that allow seed development in the absence of fertilization. Based on rescreening the first 126 putative mutants, we expect that about one third of them belong to class III, displaying components of apomicitc reproduction, for example, autonomous endosperm development and parthenogenesis.

PUBLICATIONS


In Press

Our research is based on the premise that apoptosis, a genetically controlled form of cell death, provides a natural defense against tumor development and underlies the cytotoxicity of most current anticancer drugs. Consequently, mutations that disrupt apoptotic programs can cause tumor progression and resistance to cancer therapy. Earlier studies identified the p53 tumor suppressor as an important regulator of apoptosis and demonstrated that p53 mutations could promote oncogenic transformation, tumor progression, and resistance to cytotoxic agents by reducing a cell's apoptotic potential. Our current research is aimed at elucidating how p53 promotes apoptosis and characterizing p53-independent apoptotic pathways that might function in tumor cells. We are also developing in vivo models to study the regulation of apoptosis in spontaneous mouse tumors. With Y. Lazebnik here at the Laboratory, we are investigating how p53 and other regulators of apoptosis affect activation of the apoptotic "machinery" (i.e., the molecules directly responsible for apoptotic cell death). In addition, we have begun a new direction, investigating how cells respond to ras oncogenes and the consequences of this response for multistep carcinogenesis.

Modulation of Chemosensitivity

A.V. Samuelson, At. Lin, M.E. McCurrach

One of the most confounding aspects of cancer pharmacology is the molecular basis underlying the "therapeutic index" of cytotoxic agents: Why are cancerous cells killed and most normal tissues spared? Since most anticancer agents induce apoptosis, responsive tumors must be more susceptible to apoptosis than most normal tissues. Evidence from our laboratory and elsewhere suggests that alterations in cellular susceptibility to apoptosis are tightly linked to tumorigenesis. For example, normal fibroblasts are resistant to drug-induced apoptosis but become extremely susceptible after having been made tumorigenic by coexpression of the E1A and ras oncogenes (Lowe et al., Cell 74: 954 [1993]). If these tumorigenic cells have p53 mutations, however, cytotoxic agents are no longer able to induce apoptosis efficiently. These observations suggest that tumor cell chemosensitivity is determined, at least in part, by the combined effects of oncogenic mutations on apoptosis.

Our laboratory continues to use genetically manipulated cells to mimic oncogenic changes occurring in spontaneous tumors and to modulate cellular susceptibility to apoptosis. We have shown that E1A is sufficient to promote chemosensitivity in fibroblasts. Although coexpression of a ras oncogene with E1A is able to transform primary cells (i.e., make them tumorigenic), ras does not prevent, and may in fact enhance, apoptosis.

E1A binds a series of cellular proteins, including p300/CPB, p107, p130, and pRb (the product of the retinoblastoma tumor suppressor gene). We are investigating how E1A enhances apoptosis and chemosensitivity and anticipate that this will direct us to cellular activities involved in chemosensitivity. Our results indicate that E1A's apoptotic activity requires its ability to bind and inactivate pRb (but not p107 or p130). However, this function is not sufficient; rather, a separable E1A domain cooperates with Rb inactivation to enhance cellular susceptibility to apoptosis. We are currently characterizing this other E1A function. Moreover, we are working closely with Y. Lazebnik's laboratory to characterize E1A's apoptotic activity at the biochemical level.

p53 and Apoptosis

M.E. McCurrach, T.M.F. Connor, S.W. Lowe

Inactivation of p53-dependent apoptosis promotes oncogenic transformation, tumor development, and resistance to many cytotoxic anticancer agents; however, the mechanism whereby p53 promotes apoptosis is poorly understood. p53 is a sequence-
specific binding protein that can regulate transcription. One transcriptional target of p53 that may be important for apoptosis is Bax, a cell death agonist that has homology with the anti-apoptotic Bcl-2 protein. Forced overexpression of p53 increases Bax expression in several cell types, and this increase correlates with the induction of apoptosis. In addition, the Bcl-2 and E1B 19K proteins, which can inhibit apoptosis induced by p53 overexpression, can physically associate with Bax. This raises the possibility that these oncoproteins interfere with p53-dependent apoptosis by antagonizing Bax function.

All of the studies mentioned above have relied on forced overexpression of p53 or Bax to induce apoptosis—circumstances that may not faithfully reproduce their normal activities. To determine whether bax is required for p53-dependent apoptosis, the effects of bax deficiency were examined in primary mouse embryo fibroblasts (MEFs) expressing the E1A oncogene, a setting where apoptosis is strictly dependent on endogenous p53 (Lowe et al., Cell 74: 954 [1993]). Using this approach, we demonstrated that bax can function as an effector of p53 in chemotherapy-induced apoptosis (Fig. 1) and contributes to a p53 pathway to suppress oncogenic transformation. Furthermore, we showed that additional p53 effectors participate in these processes. These p53-controlled factors cooperate with Bax to promote a full apoptotic response and their action is suppressed by the Bcl-2 and E1B 19K oncoproteins. These studies demonstrate that Bax is a determinant of p53-dependent chemosensitivity and illustrate how p53 can promote apoptosis by coordinating the activities of multiple effectors. We will continue to dissect this pathway in the coming year.

**p53-independent Apoptotic Pathways**

T.M.F. Connor, J. Polyakova, S.W. Lowe

Most cancers are treated using a combination of anticancer agents, each with a distinct primary intracellular target. This approach decreases the likelihood that mutations in any one primary drug target produce drug resistance and treatment failure. However, it is now known that agents with distinct primary targets can induce apoptosis through similar mechanisms (e.g., p53 pathway). Under these circumstances, a single mutation that disrupts the apoptotic pathway (e.g., p53 mutation) may produce resistance to multiple agents. In principle, the full benefits of combination therapy would be achieved using agents that activate distinct apoptotic pathways.

Using our simple system of fibroblasts transformed by E1A and ras, we have identified agents that induce through the p53-apoptotic pathway and
others that do not require p53 for apoptosis. As predicted, p53-dependent drugs act synergistically with p53-independent agents to induce apoptosis. Using MEFs deficient for specific genes and various cell death inhibitors, we are genetically characterizing these distinct pathways. We are also characterizing the caspases activated in each pathway, and during combination treatment (see below). We envision that this system will provide a simple model for understanding "cross-talk" between apoptotic pathways.

Caspases in Cancer
J. Polyakova, T.M.F. Connor [in collaboration with Y. Lazebnik and L. Falerio, Cold Spring Harbor Laboratory]

Proteases related to interleukin-1β converting enzyme, called caspases, are thought to be essential components of the apoptotic "machinery" (i.e., the molecules directly responsible for apoptotic cell death). The importance of apoptosis in cancer argues that components of this machinery may be tumor suppressors and/or drug sensitivity genes. Since proteolytic cleavage is essentially irreversible, caspase activation may represent the last regulated step in apoptosis. In this view, the endpoint of most, if not all, cytotoxic anticancer drugs is caspase activation. Consequently, most mutations that limit drug cytotoxicity act upstream of these proteases. If true, the caspases represent attractive drug targets.

A major goal of our laboratory is to understand how cell death regulators like p53 affect caspase activation. First, with Y. Lazebnik, we are identifying the caspases activated during p53-dependent and -independent apoptosis in the well-defined MEF system described above. Second, using viral caspase inhibitors or MEFs deficient for specific caspases, we are studying the contribution of specific caspases to various apoptotic pathways. We have shown that inactivation of p53 or overexpression of cell death inhibitors such as E1B 19K and Bcl-2 prevents caspase activation. Multiple caspases are activated during chemotherapy-induced apoptosis in MEFs, and distinct apoptotic pathways can use overlapping but nonidentical sets of caspases. These studies underscore the potential importance of caspases in cancer therapy and may ultimately provide new insights into how cell death regulators interface with the apoptotic machinery.

Multistep Carcinogenesis
S.W. Lowe, At. Lin, M.E. McCurrach [in collaboration with M. Serrano and D. Beach, Cold Spring Harbor Laboratory]

Cancer is a multistep process involving a series of genetic changes and each enhances the growth or survival of developing tumor cells. Considerable progress has been made in identifying tumor-specific mutations and how these mutations alter normal gene function. For example, mutational activation of ras results in a protein that constitutively transmits mitogenic signals, and inactivation of p53 disrupts cell cycle arrest or apoptosis. However, much less is known about how these mutations interact to produce the malignant phenotype. Why are these mutations often found in the same tumors? Why does ras mutation typically precede p53 mutation?

Perhaps the simplest model for studying the multistep nature of cancer involves oncogenic transformation of primary cells. Primary cells are genetically normal and capable of only a limited number of cell divisions in culture, after which they permanently arrest by a process known as senescence. At low frequencies, primary cells acquire mutations that disrupt senescence, allowing these variants to be established into "immortal" cell lines. Expression of oncogenic ras typically "transforms" immortal cells to a tumor-igenic state, but ras alone cannot transform primary cells. However, primary cells are made tumorigenic if ras is coexpressed with second oncogenes such as E1A (a phenomenon known as "oncogene cooperation") or when expressed in the absence of tumor suppressors such as p53 and p16. When expressed alone, these cooperating mutations (E1A, p53 loss, p16 loss) facilitate the immortalization process.

We recently demonstrated that prolonged expression of oncogenic ras in primary fibroblasts induces a permanent cell cycle arrest involving p53 and p16. Remarkably, this arrest is indistinguishable from senescence. Inactivation of either p53 or p16 prevents ras-induced arrest in rodent cells, and E1A achieves a similar effect in human cells. These observations suggest that the onset of cellular senescence can be prematurely activated in response to an oncogenic stimulus. Inactivation of this senescence program, by a cooperating oncogene or as a result of spontaneous mutation, allows proliferation to continue unabated and facilitates oncogenic transformation. Consequently, premature senescence may be an important safeguard against neoplasia.
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COLD SPRING HARBOR
MEETINGS AND COURSES
The rapid pace of advances in cell and molecular biology as well as neurobiology have made communication and dissemination of new information and technologies critical. The academic program at Cold Spring Harbor Laboratory is designed to address this need. The program comprises a wide-ranging series of postgraduate laboratory and lecture courses, workshops, large meetings, and a summer research program for undergraduates. The program now extends from a spring session of courses starting early in March through a fall session of courses ending in November.

This year, 24 courses were held at the laboratory, including 19 laboratory courses that took place in the Delbruck Laboratory and the Howard Hughes Medical Institute (HHMI)) teaching laboratories in the Beckman Neuroscience Center. Five advanced neurobiology lecture courses were held at the Banbury Conference Center.

Course instructors come from universities and research institutes around the world to teach at Cold Spring Harbor and their creativity (and endurance) really make the courses work so well. Course instructors, lecturers, and students are listed below. Several scientists who have contributed greatly by teaching courses for several years will be retiring after this year, although we know that we can count on them to give advice—and course lectures—in the future. In the spring session, these included Bruce Birren and Doug Vollrath, instructors in the course on Cloning and Analysis of Large DNA Molecules, as well as Rob Grainger and Hazel Sive, who taught Early Development of Xenopus laevis. The three-week summer courses form the core of the academic program, and several instructors who have participated in first-rate fashion over a period of years are retiring—namely, Richard Behringer and Ginny Papaioannou, who taught Molecular Embryology of the Mouse; Jim Boulter, an instructor for Molecular Cloning of Neural Genes; Ken Burtis, who taught in the Eukaryotic Gene Expression course; Ken Burtis, who taught in the Eukaryotic Gene Expression course; and Chris Kaiser, an instructor in the Yeast Genetics course, as well as Bill Mobley, who taught in the Neurobiology of Human Neurological Disease lecture course. Our thanks also go to retiring fall course instructors Paul Hough, Iris Mastrangelo, Ken Jacobson, Claire Huxley, Mike Lovett, and Tom Marr.

The courses are supported by, and would not be possible without, a series of grants from federal and private sources. The summer molecular genetics courses have been supported for many years by grants from the National Institutes of Health and the National Science Foundation. A grant from the National Institute of Mental Health that supports several of the neurobiology courses was renewed this year. A large education grant from HHMI has provided stable support for the neurobiology program and has allowed the Laboratory to begin and to expand its series of spring and fall courses. The Laboratory also has an award from the Esther and Joseph A. Klenenstein Fund for the support of neurobiology courses. As has been the case for several years, the Grass Foundation provided funds for scholarships for students in neurobiology courses. A grant from the Department of Energy has also helped in the funding of the courses in Macromolecular Crystallography. In addition, the Laboratory receives valuable support from many companies that donate supplies and lend equipment for the courses.

While sixteen meetings have been held at the Laboratory this year, the Symposium remains central. This year’s Symposium on Function and Dysfunction in the Nervous System brought together a broad array of neurobiologists to discuss recent research. Other highlights of the year included a new meeting, Cancer Genetics and Tumor Suppressor Genes, organized by Anton Berns, Terri Grodzicker, Ed Harlow, David Livingston, Carol Prives, and Bert Vogelstein. The meeting, which was over-subscribed in its first year, emphasized connections between the functions of tumor suppressor genes and their loss or mutation in different cancers. Other meetings, which are often held in alternate years, covered topics ranging from the Cell Cycle to Translational Regulation to Molecular Chaperones and the Heat Shock Response. The meetings on Genome Mapping and Sequencing and Retroviruses continue on a yearly basis. This year, two biotechnology meetings were held outside of the regular meetings season. These conferences, which started off with one held on Optical Imaging of Gene Expression and Signaling in Living Cells, are overseen by David Stewart and are expected to continue in future years. In all, over 5000 scientists attended the meetings this year. Organizers and contributors to the meetings are listed below. The sources of support
for these meetings come from the Laboratory's Corporate Sponsor program, NIH, NSF, DOE, and various corporations and foundations. Grants helped scientists at all stages of their careers to attend the conferences.

While graduate students, postdoctoral fellows, and faculty participate in the courses and meetings, the Undergraduate Research Program (URP) provides an opportunity for college undergraduates to spend 10 weeks at the Laboratory during the summer. The program, headed by Michael Hengartner, allows students to do research in the laboratories of staff scientists.

The large numbers of courses and meetings proceed with skill and efficiency, thanks to the collaborative efforts of a large number of people at the Laboratory. The staff of the Meetings Office, headed by David Stewart, Director of Meetings and Courses, coordinates the arrangements for all of the visiting scientists. This enormous job, which seems to grow every year, is carried out not only extremely efficiently, but with good humor as well. The staff, including Micki McBride, the Course Registrar, Nancy Weeks, Head Concierge, Jim Koziol, Diane Tighe, Marge Stellabotte, Andrea Stephenson, Drew Mendelson, and Michael Glaessgen, as well as staff from several other departments, are crucial to the success of the meetings and courses. These include Herb Parsons, Ed Campodonico, and the audiovisual staff; Cliff Sutkevich and his staff who set up and maintain course equipment; Edie Kappenberg, the course coordinator; Mary Horton of the Grants Office; and Lee Martin of Cold Spring Harbor Laboratory Press.
It is inherent in our nature to want to understand all that is wrong, as well as what is right in the biological systems that we study. Perhaps one of the most fascinating areas of biology where this is applicable is the nervous system. Increasingly, progress in biology is influenced by the diseases and maladies that afflict us. The decision to hold the 61st Symposium on Function and Dysfunction in the Nervous System was driven by the belief that in the near future we will learn an enormous amount about how the nervous system works by simultaneously studying the diseases that affect it. These studies, in parallel with other studies on the normal function of the nervous system, will greatly advance neurobiology in much the same way that genetics of model organisms has hastened unexpected and fundamental discoveries. The goal was to intermingle scientists interested in the study of systems in neuroscience with those trying to understand how those systems fail, so that an overview of the problems in neurological diseases could be obtained. This was a somewhat risky venture because neurobiology is as varied as biology itself, and the bringing together of investigators with diverse interests can create a hodgepodge of science that does not interest anyone. But the diseases of the nervous system are as fascinating as biology gets, and it was well worth the risk.

Some Symposia celebrate past accomplishments in fields of biology, but this one looked mostly to the future, which we hope, and even expect, is an exciting one. We anticipate that this Symposium will provide a stepping stone for future progress. The current meeting could not have been organized without the great help of several colleagues, including Eric Kandel, Stan Prusiner, Richard Axel, Conrad Gilliam, Gideon Dreyfuss, and Tony Movshon. The formal scientific program consisted of 86 speakers and 94 poster presentations. The meeting attracted 282 participants. Introductory talks on the first evening were from Michael Merzenich, Eric Kandel, Stan Prusiner, Richard Price, and Ann Pulver. The second Reginald G. Harris Lecture was delivered by Richard Axel, who presented his exciting work on olfaction. V.S. Ramachandran gave a fascinating Dorcus Cummings Lecture on the illusions of the body image to our friends and neighbors, and was widely acclaimed.

Essential funds to run this meeting were obtained from the National Institute of Neurological Disorders and Stroke and The DuPont Merck Pharmaceutical Company. In addition, the financial help from the Corporate Sponsors of our program is essential for these meetings to remain a success and we are grateful for their continued support. These sponsors are: Alza Corporation; Amgen Inc.; BASF Bioresearch Corporation; Becton Dickinson and Company; Boehringer Mannheim Corporation; Bristol-Myers Squibb Company; Chiron Corporation; Chugai Research Institute for Molecular Medicine, Inc.; Diagnostic Products Corporation; The Du Pont Merck Pharmaceutical Company; Forest Laboratories, Inc.; Genentech, Inc.; Hoechst Marion Roussel, Inc.; Hoffmann-La Roche Inc.; Johnson & Johnson; Kyowa Hakko Kogyo Co., Ltd.; Life Technologies, Inc.; Eli Lilly and Company; Merck Genome Research Institute, Oncogene Science, Inc.; Pali Corporation; The Perkin-Elmer Corporation, Applied Biosystems Division; Pfizer Inc.; Pharmacia & Upjohn, Inc.; Research Genetics, Inc.; Sandoz Research Institute; Schering-Plough Corporation; Sumitomo Pharmaceuticals Co., Ltd.; Wyeth-Ayerst Research; Zeneca Group PLC.
PROGRAM

Welcoming Remarks: Bruce Stillman

Introduction
Chairperson: W.M. Cowan, Howard Hughes Medical Institute

Neurodegenerative Diseases
Chairperson: D.J. Selkoe, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts

Addiction and Emotional Memory
Chairperson: J.R. DePaulo, Johns Hopkins University Baltimore, Maryland

Alzheimer's Disease
Chairperson: D. Price, Johns Hopkins University Baltimore, Maryland

Language and Visual Processing
Chairperson: D. Hubel, Harvard University, Boston, Massachusetts

Channels and Receptors
Chairperson: J.A. Movshon, Howard Hughes Medical Institute and Center for Neural Science, New York

Neuronal Survival and Signaling
Chairperson: H.T. Cline, Cold Spring Harbor Laboratory

Memory
Chairperson: E.R. Kandel, Howard Hughes Medical Institute, Columbia University College of Physicians & Surgeons, and New York State Psychiatric Institute

Prions
Chairperson: S.B. Prusiner, University of California, San Francisco

Neuronal Degeneration and Synapse Function
Chairperson: M.E. MacDonald, Massachusetts General Hospital, Boston

Dorcas Cummings Lecture: "Illusions of body image in neurology: What they reveal of human nature."
Speaker: V.S. Ramachandran, Dept. of Psychology and Neurosciences Program, University of California, San Diego

Perceptual Plasticity
Chairperson: L. Buck, Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts

Reginald G. Harris Lecture: "The Molecular Biology of Smell"
Speaker: A. Axel, Howard Hughes Medical Institute and Center for Neurobiology and Behavior, College of Physicians & Surgeons, Columbia University, New York

Sensory Perception
Chairperson: R. Axel, Howard Hughes Medical Institute and College of Physicians & Surgeons, Columbia University, New York

Psychiatric diseases
Chairperson: A.E. Pulver, Johns Hopkins University, Baltimore, Maryland

Neuronal Dysfunction
Chairperson: R.F. Margolskee, Mt. Sinai School of Medicine, New York, New York

Rhythms
Chairperson: C. Bargmann, University of California, San Francisco

Summary: Z.W. Hall, University of California, San Francisco and National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland
MEETINGS

Optical Imaging of Gene Expression and Signaling in Living Cells

March 15–March 17 194 participants

ARRANGED BY Steve A. Kay, Scripps Research Institute

Optical imaging technology and the development of new probes has provided biologists with unprecedented opportunities to study the molecular basis of cell organization and function. Three hundred scientists gathered over two days to discuss current achievements, but more importantly, to further explore the interface between imaging technology and studies on the expression of the genome. Intense interest has been placed on the use of green fluorescent protein (GFP) as a way of genetically encoding a fluorescent probe within the cell.

The meeting started with a keynote address by Roger Tsien, UCSD, whose laboratory has pioneered the development of color-shifted mutants of GFP. Furthermore, the use of GFP to measure protein-protein interactions via fluorescence resonance energy transfer (FRET) was discussed, as was the announcement of the development of β-lactamase as novel fluorescent marker for gene expression in living cells. Throughout the meeting many impressive talks on the use of GFP and its derivatives for studying live cell protein dynamics in Drosophila, yeast, mammalian, and plant cells were presented. For those interested in transcriptional dynamics, several talks presented the use of luciferase imaging as the only available tool for looking at rapid changes in gene transcription in living tissues and cells, including the mutation of beetle luciferases to produce new colors (K. Wood, Promega Corp.). Many exciting developments in imaging technology were presented, such as the use of two-photon excitation in confocal microscopy, which appears to be one of the most attractive methods for observing fluorescent probes in living samples. Spectacular movies of chromosome dynamics during cell division were also presented, using 3d-deconvolution microscopy techniques. Advances in NTMR microscopy were presented by Scott Fraser, of Caltech, and this method clearly promises to be a powerful tool for studying live cell events in tissues and embryos. The lectures were accompanied by a large group of posters describing a broad array of reporter gene and cellular imaging applications. Demonstrations of recent advances in CCD detector technologies were provided by the main corporate sponsor, Hamamatsu Photonics.

PROGRAM

Welcome Address: Bruce Stillman
Cold Spring Harbor Laboratory, Steve Key, University of Virginia, Charlottesville

Keynote address: Roger Tsien, University of California, San Diego

Advanced Imaging Technology and Reporter Measurement
Chairperson: Steve Kay, University of Virginia, Charlottesville

Luciferase Applications
Chairperson: Scott Fraser, Caltech, Pasadena

Applications of Green Fluorescent Protein and its Derivatives
Chairperson: Richard Day, University of Virginia, Charlottesville
The second biennial open-invitation meeting devoted to research on Zebrafish Development and Genetics was held at Cold Spring Harbor Laboratory, April 24-28, 1996. The meeting focused on the cellular, molecular, and genetic regulation of embryonic development and growth. Sessions covered: (1) neural crest and head development, (2) axis formation, (3) morphogenesis and patterning, (4) gene transfer, gene expression, and new methodologies, (5) genetic mapping, (6) inductive interactions, (7) heart, blood and other organs, (8) CNS development and (9) a satellite symposium on genomics. Additionally, a session on community issues provided an opportunity to discuss common goals of the zebrafish research community and means of fostering communication and dissemination of the information and research reagents throughout the community. There were 236 presentations.

This meeting was funded in part by the National Institute of Child Health and Human Development (a division of the National Institutes of Health) and the National Science Foundation.

**PROGRAM**

**Neural Crest and Head Development**
Chairperson: J. Eisen, University of Oregon, Eugene

**Synaptic Transmission**
Chairperson: J. Kehoe, Ecole Normale Superieure, Paris, France

**Axis Formation I**
Chairperson: C. Kimmel, University of Oregon, Eugene

**Axis Formation II**
Chairperson: M. Mullins, University of Pennsylvania School of Medicine, Philadelphia

**Morphogenesis and Patterning**
Chairperson: D. Duboule, University of Geneva, Switzerland

**Methodologies**
Chairperson: N. Hopkins, Massachusetts Institute of Technology

**Mapping**
Chairperson: J. Postlethwait, University of Oregon, Eugene

**Inductive Interactions**
Chairperson: C. Thisse, C.U. de Strasbourg, Illkirch, France

**Heart, Blood, and Other Organs**
Chairperson: M. Fishman, Massachusetts General Hospital, Harvard Medical School

**CNS Development I**
Chairperson: S. Wilson, King's College, London, United Kingdom

**CNS Development II**
Chairperson: S. Wilson, King's College, London, United Kingdom

*Perspectives on the Zebrafish Genome*
Chairperson: L. Zon, Harvard Medical School, Howard Hughes Medical Institute and Children's Hospital, Boston, Massachusetts
Molecular Chaperones and the Heat-shock Response
May 1-May 5 384 participants

ARRANGED BY
Costa Georgopoulos, University of Geneva, Switzerland
Susan Lindquist, University of Chicago
Richard Morimoto, Northwestern University

In addition to the detailed information now available on the structure and biophysical properties of the chaperoning in bacteria and in the homologue, hsp60, in mitochondria, this meeting reflected a renewed, more basic set of inquiries: how proteins are folded, how unfolded proteins are recognized, the number of substrate proteins per chaperone, and the role of nucleotide hydrolysis in chaperone activities. A fascinating session covered questions of how proteins are imported and folded in two major organelles: the endoplasmic reticulum (ER) and mitochondria. Convergence of powerful genetic and biochemical tools has revealed a role for individual chaperones and chaperone complexes in the processes of transport and folding. Studies on the question of chaperone machines focused on hsp90, the small heat-shock proteins, and the hsp70 family. The 70s and 90s do not have size restrictions and have the ability to transiently and stably interact with folding intermediates in the process of protein folding.

Questions on the regulation of chaperone gene expression addressed the bacterial transcription factors (sigma 24 and sigma 32) and their sensing mechanisms for unfolded proteins. In eukaryotes, transcriptional regulation is mediated by a family of heat shock factors that become activated in response to different types of stress and lead to the selective induction of a select number of genetic loci. The link between the stress sensing apparatus and stress protein function is in the protection of cells from molecular damage. These concepts may be relevant to diseases that involve the appearance of misfolded proteins, including Alzheimer's or prion diseases which involve cell death and in autoimmunity and infectious diseases. Manipulating the expression of stress proteins may prove beneficial to protection of tissues during trauma, including surgery and transplantation.

This meeting was funded in part by the National Science Foundation, Affinity Bioreagents, Inc., Bayer Corporation, Churchill Livingstone, F. Hoffman-LaRoche AG, Novo Nordish A/S, and StressGen.
A strong tradition of excellence was continued at this ninth Cold Spring Harbor meeting on genome mapping and sequencing. More than 260 abstracts were presented, reflecting a broad array of topics relating to the analysis of genomes from a number of different organisms. The sessions covered such areas as physical mapping of human chromosomes, multi-organismal genomics, human genetics and biology, mapping methods and technologies, gene discovery and transcript mapping, large-scale DNA sequencing, and bioinformatics.

Some of the recently established presentation formats were again utilized in an effort to provide an appropriate forum for assimilating the large amounts of incoming genome mapping and sequencing data. A poster symposium featuring the major DNA sequencing groups allowed attendees to get a glimpse of the people and strategies that will likely play key roles in sequencing the human genome. Once again, projection-style, interactive computer demonstrations in Grace Auditorium effectively showcased the critical new bioinformatics tools being developed for storing, organizing, and analyzing genomic maps and sequences.
A central theme of the meeting related to the initiation of large-scale sequencing of the human genome. Various presentations focused on the important issues relating to this critical and exciting phase of the Human Genome Project. These included the construction of sequence-ready maps, the expansion of sequence production groups, the development of sequencing automation and technologies, and the establishment of improved software for data analysis and handling.

Another major highlight of the meeting was the keynote talk given by Shirley Tilghman (Princeton University) titled “Lessons Learned, Promises Kept: A Biologist’s Eye-view of the Genome Project.” Tilghman discussed the impact of genome research on the study of biology, including the proven value of comparative genomic analysis (lessons learned) as a result of freely accessible genomic data (promises kept). A summary of this talk was published in the journal Genome Research (6:773-780, 1996).

With the anticipated developments in genome mapping and sequencing in the coming months, next year’s tenth anniversary meeting should be a particularly exciting and historic event.

This meeting was funded in part by the National Center for Human Genome Research, a division of the National Institutes of Health.
Commercial Implications of Genomics Research

May 12-May 14 118 participants

ARRANGED BY  Eric Roberts, Dillon, Read & Co. Inc.
                 David Stewart, Cold Spring Harbor Laboratory

This short conference on Commercial Implications of Genomic Research, sponsored jointly by the Laboratory and the New York investment bank Dillon, Read & Co. Inc., brought leaders in the investment and financial communities to Cold Spring Harbor to learn from current leading practitioners about the present state of knowledge and technology in genomics research. Participation was by invitation only from both East and West Coast financial communities and also from abroad, and included senior executives of biotechnology, pharmaceutical, and venture capital companies. The meeting was relatively informal in style, and all the participants, scientists as well as executives, found it to be both intellectually stimulating and very enjoyable.

Eminent keynote speakers including Jim Watson, J. Craig Venter, Eric Lander, and Robert Waterston were able to give a broad overall view of certain aspects of the field to a predominantly lay, though informed, audience. The keynote talks set the scene for discussions of particular technologies given by senior researchers from leading biotech companies (both established and start-up) who explained the science underlying their technology platforms in the context of the commercial environment. Presentations were made by Incyte Pharmaceuticals, Gene Logic, Genome Therapeutics Corporation, Exelixis Pharmaceuticals, Sequana Therapeutics, Human Genome Sciences, Darwin Molecular Corporation, Genset, Amplicon Corporation, Genetics Institute, Millennium Pharmaceuticals, GenoMed, Myriad Genetics, Mercator Genetics, Genomica Corporation and Progenitor. Two roundtable discussions on Intellectual Property (moderator Leslie Misrock, Pennie & Edmunds) and Business Models in Genomics (moderator Cynthia Robbins-Roth) were of considerable interest to the audience, while Kevin Kinsella’s dazzling postbanquet multimedia presentation provided an amusing personal and anecdotal history of the genome biotechnology industry.
This conference is intended to be the template for an annual meeting bringing relevant sections of the investment community to Cold Spring Harbor for an intensive and educational couple of days every year to learn about and discuss some topic in modern biology. We hope that this event will lead to a broader understanding of the underlying science on the part of investors, to the potential benefit of biomedical research as a whole. There may also be reciprocal benefits in bringing together leading scientists from the biotechnology industry face-to-face with the major decision-makers from the financial sectors.

We thank Dillon, Read & Co., Inc. for funding this meeting.

PROGRAM

Welcoming Comments J.D. Watson, Cold Spring Harbor Laboratory

Keynote Address J.C. Venter, The Institute for Genomic Research

Keynote Speaker E. Lander, Whitehead/MIT Center for Genomic Research

R.W. Scott; R.A. Whitfield, Incyte Pharmaceuticals, Inc.
R.Kouri, Gene Logic, Inc.
R.J. Hennessey, J.P. Richards, Genome Therapeutics Corporation
R. Barbier, J.-F. Formela, Exelixis Pharmaceuticals, Inc.
K.J. Kinsella, T.J.R. Harris, Sequana Therapeutics, Inc.
L. Misrock, Pennie & Edmunds
W.A. Haseltine, Human Genome Sciences, Inc.

G.D. Abbott, Darwin Molecular Corp.
P. Brandys, Genset, S.A.
P.G. Lowell, Amplicon Corporation
P. Gage, Genetics Institute, Inc.
C. Robbins-Roth, BioVenture Publishing

Keynote Speaker B. Waterston, Washington University

H.F. Arader, Jr., Millennium Pharmaceuticals, Inc.
P.D. Meldrum, M. Skolnick, Myriad Genetics, Inc.
E. Sigal, Mercator Genetics, Inc.
J.L. Rathmann, Genomica Corporation
D.B. Given, Progenitor, Inc.

Cell Cycle

May 15-May 19

ARRANGED BY

Fred Cross, Rockefeller University
Jim Roberts, Fred Hutchinson Cancer Research Center

The biennial cell cycle meeting is recognized internationally for its ability to bring together scientists who study cell regulation in eukaryotes ranging from yeast to humans. Although the impact of cell cycle research is being strongly felt in many other biological disciplines including developmental biology and cancer biology, this year's cell cycle meeting marked a renewed emphasis on the basic mechanisms of cell cycle regulation.

As in other years, scientists studying cell cycle regulation in yeast, Xenopus, Drosophila and mammals were well represented. Once again, the striking phylogenetic conservatism of cell cycle regulatory mechanisms was readily evident. Adding to the picture were some initial forays into cell cycle regulation in plants, a frontier that promises exciting discoveries in years to come. It was also inescapably obvious to all in attendance that the methodologic barriers that separated cell cycle studies in different organisms were finally breaking down. Yeasts no longer are the only organisms for doing genetic analyses of cell cycle regulation; impressive studies of Drosophila and mouse mutants provided major new insights into cell cycle regulatory mechanisms in higher eukaryotic organisms. Additionally, biochemical studies of cell cycle proteins in yeast are now almost on equal footing with parallel studies in other organisms. Highlighting this year's meeting was the growing emphasis on and insight into the roles of proteolysis in cell cycle regulation. The regulated destruction of cyclin B during mitosis remains the paradigm for studying ubiquitin-dependent protein turnover during the cell cycle. The discovery of the anaphase-promoting complex (APC), the elucidation of its role in the ubiquitination of cyclin B, its regulation during the cell cycle, and its possible relationship to mitotic checkpoints were all major advances described at this meeting. Equally exciting was the discovery and initial characterization of a complementary protein turnover pathway operating at the transition from G1 to S phase.
The cell cycle conference brought together 385 scientists for the presentation of 65 talks, and 184 posters. It was another landmark meeting for the cell cycle field, and the participants all continued to look forward to equally exciting meetings in future years.

This meeting was funded in part by the National Institute (a division of the National Institutes of Health) and the National Science Foundation.

PROGRAM

Keynote Address: T. Hunt, ICRF, Clare Hall Laboratories, United Kingdom

CDK Regulation
Chairperson: D. Beach, Cold Spring Harbor Laboratory

Cell Cycle Initiation
Chairperson: T. Orr-Weaver, Whitehead Institute, Cambridge, Massachusetts

Cell Cycle Completion
Chairperson: H. Pwinica-Worms, Washington University School of Medicine, St. Louis, Missouri

Cell Cycle Transcription
Chairperson: L. Breeden, Fred Hutchinson Cancer Research Center, Seattle, Washington

Cell Cycle Control
Chairperson: C. Sherr, St. Jude Children's Hospital, Memphis, Tennessee

CDK Substrates
Chairperson: R. Deshaies, California Institute of Technology, Pasadena

Coordination of Cell Cycle Events
Chairperson: T. Weinert, University of Arizona, Tucson

Cell Cycle Exit
Chairperson: S. Elledge, Baylor College of Medicine, Houston, Texas

B. Clurman, R. Sheaff

J. DiGregori, K. Knudsen, A. Koff

M. Dasso, T. Hunt

B. Kelly, B. Futcher
The annual Retrovirus has become truly international, with many foreign participants attending. Molecular aspects of both the pathogenesis and replication of these viruses were discussed. As in the last few years, a significant proportion of the contributions focused on the human pathogen, HIV. However, this comprehensive meeting is especially valuable in that it offers a unique opportunity to enhance our understanding of retrovirus molecular biology through comparing and contrasting a variety of systems.

New information was forthcoming from many levels. For example, the pathogenesis sections included not only new molecular details concerning infection with the well-studied human, primate, murine, and avian viruses, but also some of the first glimpses of pathogenesis by novel retroviruses that infect fish. In several cases, information about retroviral proteins is now available at the atomic level. Participants learned of the solution of the X-ray crystal structure of the FIV dUTP pyrophosphatase, and heard more important details concerning the structure and function of other virion proteins. They also gained a new appreciation of the ways in which detailed structural in-
formation on the retroviral enzymes, reverse-transcriptase, protease, and integrase, is facilitating the development of effective drugs to treat viral disease and also increasing our knowledge of the enzymes' mechanisms. A final session highlighted the use of exciting new technologies and new approaches to intervention, some of which may lay the groundwork for therapies of the future.

Contributions from Corporate Sponsors provided core support this this meeting.

**PROGRAM**

**Pathogenesis (nonlenti)**
Chairpersons: P. Jolicoeur, Clinical Research Institute of Montréal; J. Stoye, National Institute for Medical Research, London
Pathogenesis (lenti)
Chairpersons: M. Martin, NIAID, National Institutes of Health; R.C. Desrosiers, Harvard Medical School, New England Regional Primate Research Center

**Receptors/Entry/Uncoating**
Chairpersons: J. Young, Harvard Medical School; P. Bates, University of Pennsylvania

**Reverse Transcription**
Chairpersons: S. Hughes, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center; M. Roth, University of Medicine and Dentistry of New Jersey/RWJ Medical School

**Integration and Other Early Events**
Chairpersons: J. Leis, Case Western Reserve University School of Medicine; G. Kalpana, Albert Einstein College of Medicine

**Transcription (Tat and Tax)**
Chairpersons: J. Dudley, University of Texas, Austin; J. Coffin, Tufts University School of Medicine

**Posttranscription (Rev and CTEs)**
Chairpersons: R. Katz, Institute for Cancer Research, Fox Chase Cancer Center; M.-L. Hammarskjold, Myles H. Thaler Center for AIDS and Human Retrovirus Research and University of Virginia, Charlottesville

**Other Regulatory Proteins and Elements**
Chairpersons: M. Malim, Howard Hughes Medical Institute and University of Pennsylvania School of Medicine; J. Skowronski, Cold Spring Harbor Laboratory

**Assembly, Processing, and Egress**
Chairpersons: V. Vogt, Cornell University; R. Craven, Pennsylvania State University School of Medicine

**Interventions, Vectors, and New Technologies**
Chairpersons: G. Pavlakis, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center; H. Gottlinger, Dana-Farber Cancer Institute and Harvard Medical School

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**Cancer Genetics and Tumor Suppressor Genes**

August 14-August 18  429 participants

ARRANGED BY

Anton Berns, The Netherlands Cancer Institute
Terri Grodzicker, Cold Spring Harbor Laboratory
Ed Harlow, Massachusetts General Hospital
David Livingston, Dana-Farber Cancer Institute
Carol Prives, Columbia University
Bert Vogelstein, Johns Hopkins University

Cancer Genetics and Tumor Suppressor Genes was a new meeting at the Laboratory. It was set up to allow scientists to discuss their latest results on different aspects of cancer cell biology and to allow for extensive crosstalk concerning ideas and methodologies. The conference was, in fact, over-subscribed, with 429 scientists meeting to present more than 300 talks and posters. Launched by a keynote address by Richard Klausner, Director of the National Cancer Institute, the meeting presented an extensive range of work: from location and nature of mutations in different tumors to properties of the p53 tumor suppressor gene. Many talks dealt with growth control of cancer cells, and areas that received much attention included control of the cell cycle, apoptosis, and transcriptional regulation of and by tumor suppressor genes. Animal models have provided much information about tumorigenesis, and one session dealt with "knock-out" and transgenic mouse models. The wide range of studies in this area was emphasized by presentations that dealt with tumor progression, angiogenesis, and metastasis. This new meeting was off to an exciting start and all sessions found the lecture and poster halls packed with extensive discussions and exchanges of information.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.
PROGRAM

Animal Models
Chairpersons: A. Berns, The Netherlands Cancer Institute, Amsterdam; A. Bernstein, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

Cell Cycle
Chairpersons: E. Harlow, Massachusetts General Hospital Cancer Center, Boston; G. Vande Woude, NCI-Frederick Cancer Research and Development Center, Maryland

Tumor Cell Biology
Chairpersons: B. Vogelstein, Johns Hopkins University, Baltimore, Maryland; E. Fearon, University of Michigan Medical Center, Ann Arbor

Transcriptional Regulation
Chairpersons: D. Livingston, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts; C. Prives, Columbia University, New York, New York

p53
Chairpersons: A. Levine, Princeton University, Princeton, New Jersey; C. Harris, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Cancer Genetics
Chairpersons: B. Ponder, CRC Human Cancer Genetics Research Group, Addenbrookes Hospital, University of Cambridge, United Kingdom; L. Strong, University of Texas System Cancer Center, Houston

Chromosome Behavior and Stability
Chairperson: C. Greider, Cold Spring Harbor Laboratory

Apoptosis
Chairpersons: E. White, Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, New Jersey; R. Kolodner, Div. of Human Cancer Genetics, Dana-Farber Cancer Institute, Boston, Massachusetts
This year's meeting was enhanced by invited scientists who chaired each of the sessions. Given the diversity of session topics, their introductory remarks, providing general background information and outlining unanswered questions in the field, were particularly valuable.

As usual, it required several sessions to cover multiple aspects of transcription and gene regulation, but the increased activity in cell biology was notable. In the Signaling and Global Circuits and Cell Division and Development sessions, speakers used fluorescence microscopy to examine how cell-type-specific gene expression is established during sporulation in *B. subtilis*, and how the sites of cell division are determined in *E. coli*. Then, in the Cell Surfaces & Secretion session, we got the first insight into the role the eukaryotic Signal Recognition Particle homolog plays in *E. coli*.

Sophisticated genetic and biochemical approaches were used to examine the architecture and reaction mechanism of enzymes. One provided direct visualization of a repressor protein sliding along a DNA molecule, looking for its binding site. Two elegant studies described a built-in checkpoint for the activity of a transposase; since a subunit bound to one site catalyzes recombination at the other site, and subunits bound to two different sites form a single, shared catalytic domain, catalysis can occur only when the distant sites have been brought together in the appropriate configuration. Another presentation concerned the mechanism of protein splicing. Protein splicing may be an arcane biological oddity, but the details of the mechanism, and the application to a new system for protein purification based on the cleavage reaction, generated a great deal of excitement.

The scientific community had been puzzled for several years by reports that seemed to support the Lamarkian idea that useful or "adaptive" mutations occur preferentially. A talk that presented data contradicting this interpretation, along with an explanation for why "nonselected" (i.e., not useful) mutations had not been detected previously, was enthusiastically received.

This meeting was funded in part by the U.S. Department of Energy.
The Mouse Molecular Genetics conference, held in alternate years at the EMBL, Heidelberg, attracted the usual capacity crowd of international researchers. This meeting occupies a singularly important niche, bringing together a large cohort of people working in diverse areas of mouse biology. This year’s conference was no exception, covering topics from genetic approaches (both traditional and new wave) to tumorigenesis and behavior.

The meeting was organized into eight sessions, each composed of two longer, invited presentations in addition to eight shorter talks selected from the abstracts. The conference was rounded out by the inclusion of a record-breaking 280 posters, presented in two afternoon sessions, and made possible by the provision of additional tented accommodation. This volume of information considerably enhanced the scientific content of the meeting, even if the average participant was left "staggering and "frazzled" by the wealth of new, and largely unpublished data.

Highlights of the platform presentations included important new mechanistic insights into the process of X-chromosome inactivation, and the roles played by components of the activin signaling pathway in mesoderm formation in the early embryo. A number of classic mouse mutations, includ-
ing staggerer, beige and fused, have yielded up their molecular identities. Finally, a number of presentations included significant, and long-awaited advances in strategies for generating conditional mutations and binary systems to study cell fate. These topics, and many more, made for lively discussion among the 430 participants. In all, a very stimulating and interactive meeting.

This meeting was funded in part by the National Science Foundation; and the National Institute of Child Health and Human Development, the National Cancer Institute, and the National Institute of Neurological Disorders and Stroke, branches of the National Institutes of Health.

PROGRAM

Genetics I
Invited Speaker: P. Avner, Institut Pasteur, Paris, France
Chairperson: N. Hastie, Western General Hospital, Edinburgh, United Kingdom

Patterning
Invited Speaker: L. Buck, Howard Hughes Medical Institute and Harvard University Medical School, Boston, Massachusetts

Information Coding in the Olfactory System
Chairperson: C. Tabin, Harvard University Medical School, Boston, Massachusetts

Tumorigenesis
Invited Speaker: R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

DNA Methylation, Imprinting, and Cancer
Chairperson: A. Berns, The Netherlands Cancer Institute, Amsterdam

Embryogenesis
Invited Speaker: M. Yanagisawa, Howard Hughes Medical Institute and University of Texas Southwestern Medical Center, Dallas

Endothelin Pathway in Neural Crest Development
Chairperson: J. Rossant, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, and University of Toronto, Canada

Signals and Receptors
Invited Speaker: R. Derynck, University of California, San Francisco
*Epithelial immaturity in EGFR-/- mice*
Chairperson: S. Korsmeyer, Washington University School of Medicine, St. Louis, Missouri

Neural Development
Invited Speaker: M. Bronner-Fraser, California Institute of Technology, Pasadena
*Formation of Neural Crest*
Chairperson: M. Placzek, MRC, London, United Kingdom

Genetics II
Invited Speaker: J. Takahashi, Northwestern University, Evanston, Illinois
*Genetics of Circadian Clocks in Mice*
Chairperson: F. Costantini, Columbia University College of Physicians & Surgeons, New York

Organogenesis
Chairperson: C. Wright, Vanderbilt University School of Medicine, Nashville, Tennessee
The Translational Control meeting, yet again, brought a significant increase in the number of participants and of abstracts, indicative that interest in these topics is still blossoming and testifying to the paramount importance of this meeting as the major international forum of the field. As in previous years, animal, plant and viral systems were very well represented, but, with a few notable exceptions, those interested in prokaryotic systems seem to have sadly abandoned this meeting, which has the unfortunate consequence that ribosome structure and function was also poorly represented. On the positive side, there was a very welcome increase in the number of presentations dealing with developmental topics, with a particular upsurge in work on *Drosophila* embryogenesis. Of particular significance is the growing appreciation that embryogenesis involves not just specific mRNA localization but also a temporal control of maternal mRNA translation.

A major new area of discussion at this meeting was the structure, function, and regulation of the eIF4F holoenzyme complex. Cleavage of the eIF4G component by picornavirus proteases indicates that it serves a bridging function to bring together a number of important initiation factors: eIF4E, the cap-binding protein, binds to the N-terminal one-third of eIF4G; eIF3 binds to the central region; and the helicase eIF4A interacts with the C-terminus, at least in the case of mammalian eIF4G. In addition, at least in the case of yeast, the N-terminus of eIF4G interacts with Pablp, provided that the latter is bound to poly(A), which would promote physical interaction between the two ends of the mRNA. Consistent with this is the fact that the normal pathway of mRNA degradation in yeast involves first the shortening of the poly(A) tail, then decapping, and finally degradation by a 5' → 3' exonuclease, but in mutants lacking Pablp, the decapping occurs prematurely without the need for poly(A) tail shortening.

An unexpected finding is that mammalian cells have what seems to be an eIF4G decoy, which shares homology with the C-terminal two-thirds of eIF4G, and appears to act as a negative regulator of initiation. In livers of transgenic animals over-expressing apolipoprotein B-editing enzyme, this decoy is ablated as a result of hyper-editing of its mRNA, and the outcome is rampant hepatocarcinoma. A related topic that was barely in its infancy at the last meeting is the impact of...
the major signaling pathway on translation initiation exerted via 4E-BPI, a protein which binds the cap-binding initiation factor eIF4E and sequesters it from entry into the eIF4F holoenzyme complex. Phosphorylation of 4E-BPI by the rapamycin-sensitive pathway inhibits its interaction with eIF4E, thus liberating the cap-binding protein for entry into the eIF4F holoenzyme complex, and hence increasing initiation rates.

While the above may be regarded as the highlights, in a short report of this nature it is not possible to mention numerous examples of excellent work reported in other areas, and the meeting certainly left the impression of good progress being made on many different fronts.

This meeting was funded in part by RiboGene, Inc., ICN Pharmaceuticals, Inc. Promega Corp., and Novagen, Inc.

PROGRAM

5' UTR Functions  
Chairperson: A.P. Geballe, Fred Hutchinson Cancer Research Center, Seattle, Washington
Poly(A), PABP AND 3' -5' Crosstalk  
Chairperson: L. Gehrke, Massachusetts Institute of Technology, Cambridge
Developmental Regulation  
Chairperson: J. Kimble, University of Wisconsin, Madison
Regulation of Initiation  
Chairperson: L.S. Jefferson, Pennsylvania State University, Hershey

Workshop: Factors and Mechanisms  
Chairperson: J.W.B. Hershey, University of California, Davis
Elongation and Termination  
Chairperson: J.F. Atkins, University of Utah Medical School, Salt Lake City
mRNA Stability and Turnover  
Chairperson: L.E. Maquat, Roswell Park Cancer Institute, Buffalo, New York
Initiation Factors: Structure and Function  
Chairperson: K.S. Browning, University of Texas, Austin
RNA-Protein Interactions  
Chairperson: M.W. Hentze, EMBL, Heidelberg, Germany

Molecular Approaches to the Control of Infectious Diseases

September 9-September 13  157 participants

ARRANGED BY  
Fred Brown, USDA, Plum Island Animal Disease Center
dennis Burton, Scipps research Institute
Peter Doherty, St Jude Children's Research Hospital
John Mekalanos, Harvard University Medical School
Erling Norrby, Karolinska Institute, Sweden

The 14th Annual Meeting on New Vaccines, now called Molecular Approaches to the Control of Infectious Diseases was held on September 9-13, 1996. As in previous years, the program had a balance of talks describing the basic concepts underlying the eliciting of immunity against infectious agents and those dealing with the application of these concepts to the design of new vaccines. The importance of structural studies was illustrated by John Skehel's talk on influenza hemagglutinin and by the presentations of Don Wiley and Luc Teyton on the interaction of antigens of with T-cell receptors. An intriguing talk by Marc van Regenmortel described the immunogenic activity of peptides synthesized from D-amino acids by aligning the residues in the reverse order
from that on the parent L-peptide. In such all-D-retropeptides, the side chains are oriented as in the
parent L-peptide; consequently, antibodies raised against the L- or all-D-retro forms cross-react
with both structures. In a practical application of this approach, the all-D-retropeptide correspond-
ing to the immunogenic loop of foot-and-mouth disease virus was shown to elicit protective
neutralizing antibodies. Pasteur and Landsteiner would have appreciated this approach.

It has been shown in several animal models that injection of the DNA coding for immunogenic
proteins and epitopes of infectious agents can evoke immunity. The mechanisms underlying these
observations are not well understood, but several papers presented indicated that these questions
are now being investigated. At the practical end of the spectrum Erling Norrby’s historical talk on
vaccination against measles illustrated how our understanding of the structure of the virus and its
immunogenic proteins has answered questions from the past and pointed the way forward in the
efforts being made to eradicate the disease.

As in the previous two meetings, the Albert Sabin Foundation lecture was included in the pro-
gram. On this occasion, Joe Melnick, who contributed so much to our understanding of
poliomyelitis infection and its control by vaccination, gave a historical review of our knowledge of
the virus. Salk and Sabin are the names that are remembered, but Joe and others made important
contributions in the 1950s which led to the two highly successful vaccines.

This report is also a farewell message. Since this will be the last year in which Cold Spring Har-
bor Laboratory will host a program on new vaccines, it is appropriate that we mention Bob
Chanock and Richard Lerner, the founders of the meeting in 1983, and the organizers of sub-
sequent meetings, Harry Ginsberg, Erling Norrby, John Mekalanos, Dennis Burton, and Peter
Doherty. It has been a pleasure and an education to work with them and the staff at Cold Spring
Harbor Laboratory, particularly Dorothy Brown, Mary Cozza, and Inez Sialiano in Publications, Micki
McBride in the Meetings & Courses Office, and Herb Parsons of Public Affairs, who always ensured
that the meetings ran like clockwork. This meeting was funded in part by Pharmacia LKB Biotech-
nology.
The annual meeting on Molecular Biology of Hepatitis B Viruses was held at the Cold Spring Harbor until 1989 and this year marked the return of this international meeting to Cold Spring Harbor. Many exciting new developments were presented on a variety of topics focusing on a virus that induces chronic infections that represent the fourth leading cause of deaths due to infectious diseases worldwide. A much greater emphasis is being placed on the interaction of the virus and viral proteins with the host than was in recent years. Many new developments were reported regarding processing and intracellular transport of viral transcripts and potentially involved host factors. New methods were developed to better define the sequence and structural requirements for the epsilon PNA sequence, which is involved in encapsidation of viral PNA as well as protein-primed initiation of reverse transcription. Data demonstrating the interaction of cellular chaperone proteins with the reverse transcriptase and the reconstitution of polymerase activity from individually expressed domains were presented. Sequences involved in later steps of genomic replication are beginning to be defined. Although the cell surface receptor for HBV still has not been identified, a cellular protein that may represent a portion of the receptor complex has been characterized, and additional potential receptor candidates are being examined. Studies on the membrane topology and amino acid sequences within the large-envelope protein needed for receptor binding were presented. The mechanism of transcriptional activation by the X protein continues to be highly complex due to the numerous interactions this protein has with cellular pathways. Studies in transgenic and knockout mice demonstrated that both perforin and FasL pathways are required for cytolytic viral clearance, but that noncytolytic viral elimination by cytokines may be more or equally important. Although new and promising nucleoside analogs were described for treatment of chronic infections, the observation that long-term treatment with nucleoside analogs does not result in clearance of covalently closed circular DNA from the nucleus indicates the difficulty of overcoming this obstacle in order to clear chronic infections.
This meeting was funded in part by a grant from the National Institute of Allergy and Infectious Diseases and the National Cancer Institute, both branches of the National Institutes of Health. Contributions from the following companies were provided to this meeting for travel grants for young scientists: Glaxo Wellcome Inc., Merck Research Laboratories, Schering Plough Research Institute, Bayer AG, Appolon Inc., Roche Products Ltd., Abbott Laboratories, Behringwerke AG, and Boehringer Ingelheim GmbH.

PROGRAM

Transcription
Chairpersons: D.L. Johnson, University of Southern California; Y. Shaul, Weizmann Institute of Science, Rehovot, Israel

Replication
Chairpersons: D. Loeb, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; P. Marion, Stanford University School of Medicine, California

Structural Proteins/Receptors
Chairpersons: W. Gerlich, Justus Liebig University, Giessen, Germany; J. Summers, University of New Mexico Cancer Research and Treatment Center, Albuquerque

Seminar
M. Mathews, Cold Spring Harbor Laboratory
Interactions Between Viruses and the Host Translation System

Regulatory Proteins
Chairpersons: H. Schaller, ZMBH, University of Heidelberg; K. Koike, Cancer Institute, Tokyo, Japan

HCC
Chairpersons: C. Rogler, Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Bronx, New York; M.A. Buendia, Institut Pasteur, Paris, France

Variants/HDV
Chairpersons: H. Will, Heinrich-Pette Institut, Hamburg, Germany; J.L. Gerin, Georgetown University Medical Center, Rockville, Maryland

Immunology/Pathogenesis
Chairpersons: F. Chisari, Scripps Research Institute, La Jolla, California; A. Jilbert, University of Adelaide, Australia

Workshop I: Receptors
Moderator: J. Pugh, Fox Chase Cancer Center, Philadelphia, Pennsylvania

Workshop II: WHV
Moderator: P. Cote, Georgetown University, Rockville, Maryland

Antivirals
Chairpersons: M. Melegari, Massachusetts General Hospital Cancer Center and Harvard Medical School, Charlestown; F. Zoulim, INSERM U271, Lyon, France

Gene Therapy
September 19-September 25 463 participants

ARRANGED BY
Theodore Friedmann, University of California, San Diego
Richard Mulligan, Massachusetts Institute of Technology
Gary Nabel, University of Michigan
David Weatherall, University of Oxford

Cold Spring Harbor Laboratory hosted the third in its series of biennial meetings on human gene therapy. Despite discouraging publicity accompanying a number of inconclusive clinical studies
published since the 1994 meeting, there has been an explosion of interest and progress in the field, and a growing conviction that truly beneficial clinical applications of gene transfer technologies will soon appear. This optimism was reflected by the fact that the meeting was very heavily oversubscribed. Of the many meetings on gene therapy, Cold Spring Harbors remains the only one with an abstract-driven format and, therefore, continues to be the most accessible to the broad gene therapy community, including students, fellows, and junior and senior investigators. While results and progress in a number of ongoing clinical trials were presented, the program emphasized the molecular genetics and cell biology issues underlying approaches to human gene therapy. The organizers are convinced that interest and progress in human gene therapy will grow enormously in the very near future and that further meetings at Cold Spring Harbor will continue to satisfy an important need in the scientific and medical communities.

This meeting was funded in part by the National Institute of Child Health and Human Development; National Cancer Institute; National Institute on Aging; National Institute of Diabetes and Digestive and Kidney Diseases; National Institute on Deafness and Other Communication Disorders; National Institute of Dental Research; National Institute of Mental Health; and the National Institute of Neurological Disorders and Stroke; all branches of the National Institutes of Health.

PROGRAM

Hematopoietic
Chairperson: D. Weatherall, University of Oxford, United Kingdom

Cancer I
Chairperson: G. Nabel, Howard Hughes Medical Institute, University of Michigan, Ann Arbor
National Gene Vector Laboratory Report
Chairperson: K. Cornetta, Indiana University, Indianapolis

Cancer II
Chairperson: A. Nienhuis, St. Jude Children’s Research Hospital, Memphis, Tennessee

Viral Vectors
Chairperson: R. Mulligan, Massachusetts Institute of Technology, Cambridge

Non-viral Vectors
Chairperson: M.A. Liu, Merck Research Laboratories, West Point, Pennsylvania

Aids
Chairperson: I. Verma, Salk Institute, La Jolla, California

Regulatory Issues
Chairperson: N. Wivel, University of Pennsylvania, Philadelphia

Disease Models
Chairperson: T. Friedmann, University of California, San Diego, La Jolla

Emerging Technology
Chairperson: J. Leiden, University of Chicago, Illinois

Learning and Memory

October 2-October 6 145 participants

ARRANGED BY
Per Anderson, University of Oslo
Eric Kandel, Columbia University
Richard Thompson, University of Southern California
Susumu Tonegawa, Massachusetts Institute of Technology
The third biennial meeting on Learning and Memory consisted of platform and poster presentations and discussions of current research and concepts in the biological basis of memory. Study of the biological bases of learning and memory is a most exciting field in neuroscience. Progress is currently very rapid, particularly at molecular, cellular, neural systems, and computational levels. Commonalties of mechanisms appear to be emerging from studies of gene expression, synaptic plasticity (LTP and LTD) and processes of memory storage in invertebrate and vertebrate systems. In particular, similar changes in the properties of membrane channels and intracellular second messenger systems were identified in a number of different neural systems in both vertebrate and invertebrate preparations. Discussions ranged from brain systems of memory in humans through brain circuits and systems of memory, to brain plasticity in development, basic processes of learning and memory, mechanisms of synaptic plasticity, e.g., long-term potentiation (LTP) and long-term depression (LTD), biophysical and molecular substrates of synaptic plasticity, to genetic approaches to mechanisms of memory.

A special focus of the meeting was on the use of mutant and transgenic mice to identify the roles of gene expression of particular enzyme systems in synaptic plasticity and memory. The 'gene knockout' approach is new and very promising. There was much discussion about the possibility of developing localized, inducable, reversible knockout preparations.

The meeting was funded in part by the National Science Foundation.

PROGRAM

Higher Nervous System Function
Chairperson: L. Ungerleider, Laboratory of Brain and Cognition, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

Brain Circuits and Systems of Memory
Chairperson: A. Graybiel, Dept. of Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge

Basic Processes of Learning and Memory
Chairperson: Y. Dudai, Weizmann Institute of Science, Rehovot, Israel

Transition from Short- to Long-term Memory
Chairperson: F. Nottebohm, Rockefeller University, New York

Computational Models of Learning and Memory
Chairperson: E. Marder, Volen Center and Biology Dept., Brandeis University, Waltham, Massachusetts

Mechanisms of Synaptic Plasticity—LTP, LTD, and Other
Chairperson: T. Carew, Dept. of Psychology, Yale University, New Haven, Connecticut

Biophysics and Molecular Biology of Synaptic Plasticity and Memory
Chairperson: J. Byrne, Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston

Genetic Approaches to Mechanisms of Memory
Chairperson: A. Silva, Cold Spring Harbor Laboratory

Neurotrophins, Brain Plasticity, and Development
Chairperson: M.E. Hatten, Rockefeller University, New York
The technique of differential display of mRNA following reverse transcription and polymerase chain reaction (PCR) amplification was described initially in 1992 by Liang and Pardee. The closely-related procedure of RNA fingerprinting by arbitrarily primed PCR (RAP-PCR) was developed at the same time by McClelland and Welch. Cold Spring Harbor opted to host a meeting focusing on the broad applicability of these related display techniques in a rapidly maturing field. In the first session, Arthur Pardee and Michael McClelland described the development of differential display and RAP-PCR. Next came sessions devoted to advances in the methodologies of differential display, including techniques for reducing the false positives and for rapidly confirming that differentially displayed cDNA fragments are differentially expressed in the tissues or cells of interest. Several groups reported respectively on nonradioactive differential display and on techniques allowing the differential display of cDNA fragments in the 1.0–2.0 Kb range, thus increasing the likelihood of obtaining information on coding sequence. Two further sessions were devoted to applications of these techniques to an amazingly broad range of biological problems that included forestry (the study of wind stress in poplar trees), hematopoiesis, neurobiology, cardiology, cancer, immunology, psychiatry, neuroendocrinology and many others. The final session focused on techniques emerging from the general approach used in differential display and RAP-PCR, including the display of 3'-restriction fragments and expression monitoring by hybridization to high-density oligonucleotide arrays. Highlights of the meeting included advances in differential display technology (differential display of long cDNA fragments) and the emergence of new techniques based on the concepts of differential display and RAP-PCR, including suppression subtractive hybridization and the display of 3'-restriction fragments of cDNA. The most dramatic presentation at the meeting was the introduction of the DNA chip-screening technologies, which many believe will replace differential displays and related technologies as the human genome project is completed.

Major funding for the meeting was provided by Beckman Instruments Inc. and Genomyx Corporation (in corporate partnership with Beckman Instruments Inc.). Additional financial support was also provided by Clontech Laboratories, Inc., GenHunter Corporation, Merck and Company, Inc., Millennium Pharmaceuticals, NEN Life Science Products Division of DuPont, Qiagen Inc., and SmithKline Beecham Pharmaceuticals.
PROGRAM

Methodology I
Chairpersons: P. Liang, Vanderbilt Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee; M. McClelland, Sidney Kimmel Cancer Center, San Diego, California

Methodology II
Chairpersons: M. Erlander, R.W. Johnson Pharmaceutical Research Institute, San Diego, California; J.A. Winkles, American Red Cross, Holland Laboratory, Rockville, Maryland

Applications I
Chairpersons: T. Ito, Human Genome Center, Institute of Medical Sciences, University of Tokyo, Japan; J. Guimares, DNAX Research Institute, Palo Alto, California

Applications II
Chairpersons: G. Theissen, Max-Planck-Institut für Züchtungsforschung, Cologne, Germany; M.E. Russell, Harvard School of Public Health, Boston, Massachusetts

Emerging Techniques
Chairpersons: S.A. McCarthy, DNAX Research Institute, Palo Alto, California; S. Weissman, Yale University School of Medicine, New Haven, Connecticut
The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Advanced Genome Sequence Analysis

March 20–April 2

INSTRUCTORS

Chen, Ellison, Ph.D., Perkin Elmer Corporation
Gibbs, Richard, Ph.D., Baylor College of Medicine
McCombie, W. Richard, Ph.D, Cold Spring Harbor Laboratory
Wilson, Richard, Ph.D., Washington University School of Medicine

ASSISTANTS

Dear, Simon, The Sanger Center, United Kingdom
Heiner, Cheryl, Applied Biosystems, Inc.
Hoffman, Jane, Cold Spring Harbor Laboratory
James, Carolyn, University of Manchester, United Kingdom
Johnson, Doug, Washington University School of Medicine
McPherson, John, Washington University School of Medicine
Muzny, Donna, Baylor College of Medicine
Till, Sally, Cold Spring Harbor Laboratory
Zhu, Lin, Sequanna Therapeutics

Recent advances in the automation of DNA sequencing have opened new possibilities for the analysis of complex genomes at the DNA sequence level. This 2-week course provided intensive training in this rapidly evolving field. The course emphasized techniques and strategies for using automated sequences to sequence large, contiguous genomic regions. Students carried out all of the steps in the sequencing process from preparing cosmid DNA to computer analysis of the finished sequence. Topics included subclone library generation, large-scale template purification, sequencing reactions, gel analysis on automated sequencers, sequence assembly, gap filling, and conflict resolution. Students worked in groups to sequence a large region of DNA. In last year’s course, a 45-kb cosmid was sequenced (GenBank accession #U23729). Through this process the students were trained in crucial project and data management techniques. A series of lecturers discussed their applications of these techniques as well as alternate strategies for high-speed-automated DNA sequencing.

Last year’s speakers included C.T. Caskey, A. Dusterhoft, T. Marr, D. Smith, R. Smith, F.W. Studier, B. Roe, R. Weiss, and J.D. Watson.
PARTICIPANTS
Clark, M., B.S., Ph.D., University of Cambridge School of Medicine, United Kingdom
Coleman, D., B.S., University of Oxford, United Kingdom
Dumanski, J., M.D., Ph.D., Karolinska Institute, Sweden
Goff, S., B.S., Ph.D., CIBA, North Carolina
Hitte, C., B.S., M.S., CNRS, France
Kraemer, E., B.A., Ph.D., Washington University, St. Louis
Little, R., B.S., M.S., Eli Lilly & Co., Indiana
Ma, J., B.S., M.S., Millenium Pharmaceutical, Inc., Massachusetts
Khong, N.V., M.S., D.V.M., Vrije University, Brussels
Podlutsky, A., B.S., M.S., Karolinska Institute, Sweden
Reichard, R., B.S., M.S., SmithKline Beecham, Pennsylvania
Schmuck, K., B.S., Ph.D., Sandoz Pharmaceutical, Ltd., Switzerland
Touchman, J., B.A., Ph.D., National Institutes of Health
Van Geel, M., M.S., Roswell Park Cancer Institute, New York
Vitale, D., B.S., Universita "Federico II," Italy

SEMINARS
Chen, E., Perkin-Elmer Corporation. Ordered shotgun sequencing: Strategy to integrate mapping and large-scale sequencing.
Gibbs, R., Baylor College of Medicine. Large-scale sequencing strategies for the human X chromosome.
Mathies, R., University of California, Berkeley. Energy-transfer fluorescent primers for DNA sequencing and analysis.
Metzger, M., Baylor College of Medicine, Houston. Development of novel fluorescent dyes for quantitation of heterogeneous HIV-1 populations using automated DNA sequencing.
Roe, B., University of Oklahoma, Norman. Sequencing the human genome: Lessons from the first 3 million bases.
Schwartz, D., New York University. New methods for optimal mapping of large DNA clones.
Uhlen, M., Royal Institute of Technology, Stockholm. Automated sequencing of PCR products.
Wilson, R., Washington University, St. Louis. Genome analysis by large-scale DNA sequencing.
Cloning and Analysis of Large DNA Molecules

April 10–23

INSTRUCTORS
Abderrahim, Hadi, M.D., Ph.D., Centre d’Etudes du Polymorphism Humain, France
Birren, Bruce, Ph.D., Whitehead Institute/MIT Center for Genome Research
Riethman, Harold, Ph.D., Wistar Institute
Vollrath, Douglas, M.D., Ph.D., Stanford University

ASSISTANTS
Gros, Isabelle, Centre d’Etudes du Polymorphism Humain, France
Negorev, Dmitri, Medical College of Pennsylvania
Nguyen, Michael, Whitehead Institute/MIT Center for Genome Research

This course covered the theory and practice of manipulating and cloning high-molecular-weight DNA. The course focused on the use of yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) cloning systems for library construction and techniques of pulsed-field gel electrophoresis (PFGE). Lectures and laboratory work included an introduction to yeast genetics, the isolation and manipulation of high-molecular-weight DNA from a variety of sources, and preparative and analytical PFGE. Clones were produced and characterized by several approaches including library screening, contig assembly, long-range restriction and RecA-assisted restriction endonuclease (RARE) cleavage mapping, and recovery of YAC ends. Lectures by outside speakers on topics of current interest supplemented the laboratory work.
PARTICIPANTS
Araya, Z., B.S., Uppsala University, Sweden
Cai, Li, B.S., Ph.D., Harvard Medical School /Children's Hospi-tal
Crooijmans, R., B.S., Wageningen Agricultural University, The Netherlands
Diaz-Perez, S., B.S., Ph.D., University of California, Los Angeles
Fielding, R., B.S., Chromaxome Corporation, San Diego, California
Hubner, N., M.D., University Hospital Benjamin Franklin, Germany
Koike, G., M.D., Ph.D., Medical College of Wisconsin
Mosser, J., B.S., Ph.D., CNRS, UPR 41, France
Nielsen, L.B., M.D., University of California, San Francisco
Santos, C., B.S., Ph.D., Universidad Publica De Navarra, Spain
Soong, T.-W., B.S., Ph.D., National University of Singapore, Singapore
Stoilov, I., M.D., University of Connecticut Health Center
Suchyta, S., M.S., Michigan State University
Van Lingen, B., B.S., Tufts University
Vantoai, T., B.S., Ph.D., United State Department of Agricultural Research
Wiltshire, T., B.S., University of Tennessee

SEMINARS
Doggett, N., Los Alamos National Laboratory. Physical mapping of chromosome 16: Methods, results, and lessons learned.
de Jong, P., Rosewell Park Cancer Institute, Buffalo, New York. Preparation of the PEC and BAC libraries for the human genome.
Gnirke, A., Mercator Genetics, Inc., Menlo Park, California. Distance calibration of YAC-contig and STS maps by RARE cleavage.

Protein Purification and Characterization

April 10–23

INSTRUCTORS
Burgess, Richard, Ph.D., University of Wisconsin, Madison
Courey, Albert, Ph.D., University of California, Los Angeles
Lin, Sue-Hwa, Ph.D., University of Texas/M.D. Anderson Cancer Center
Mische, Sheenah, Ph.D., Rockefeller University

ASSISTANTS
Arthur, Terry, University of Wisconsin, Madison
Burke, Thomas, University of California, San Diego
Earley, Karen, University of Texas/M.D. Anderson Cancer Center
Gharahdaghi, Farzin, Rockefeller University
Grabski, Tony, University of Wisconsin, Madison
Valentine, Scott, University of California, Los Angeles

This course was intended for scientists who were not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue; (2) a sequence-specific, DNA binding protein; (3) a recombinant protein overexpressed in E. coli; and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques were employed including precipitation by salts, pH, and ionic polymers; ion exchange, gel filtration, hydrophobic interaction, and reverse-phase chromatography; lectin affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis and electroblotting; and high-performance liquid chromato-
graphy. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized including immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization rather than on automated instrumental analysis. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology. Guest lecturers have included S. Darst, J. Edsall, G. Guidotti, J. Kadonaga, A. Kornberg, D. Marshak, N. Pace, G. Rose, J. Rothman, and B. Stillman.

PARTICIPANTS
Benito, M.-I., B.A., Stanford University
Brandriss, M., B.A., Ph.D., New Jersey Medical School
Coffey, R., B.A., M.D., Vanderbilt University Medical Center
Danziger, R., B.A., M.D., Columbia University College of Physicians & Surgeons
Hoskins, S., B.S., Ph.D., City College of New York
Kaplan, M., B.A., Stanford University
Moore, S., B.S., Ph.D., National Cancer Institute, Frederick Cancer Research Center
Nagel, T., M.D., University of California, San Francisco
Peris, M., B.S., University of California, Los Angeles
Romanowski, P., B.S., University of Cambridge, United Kingdom
Schiller, N., B.S., Ph.D., University of California, Riverside
Todd, A., B.S., McGill University, Canada
Valenzuela, M., B.S., Ph.D., Meharry Medical College
Van Lohuizen, M., B.S., Ph.D., Netherlands Cancer Institute, The Netherlands
Visa, N., B.S., Ph.D., Karolinska Institute, Sweden
Xue, D., B.S., Ph.D., Massachusetts Institute of Technology

SEMINARS
Burgess, R., University of Wisconsin, Madison. Overview of protein purification, immunoaffinity purification.
Courey, A., University of California, Los Angeles. Transcriptional regulation of dorsal/ventral pattern formation in Drosophila.
Darst, S., Rockefeller University. Preparation and analysis of proteins for structural studies: Applications to E. coli RNA polymerase.
Edsall, J., Harvard University. Perspectives on 70 years of protein purification.
Guidotti, G., Harvard University. Can insulin receptor function as a monomer?
Kadonaga, J., University of California, San Diego. Transcription, chromatin, and purification of nuclear proteins.
Marshak, D., Osiris Therapeutics, Baltimore, Maryland. Phosphorylation and regulation of cell growth.
Lin, S.-H., University of Texas/M.D. Anderson Cancer Center. Purification of membrane proteins and cell adhesion molecule, C-CAM.
Mische, S., Rockefeller University. Microanalytical protein preparation and characterization.
Pace, N., Texas A&M University. Protein folding and stability.
Rothman, J., Memorial Sloan-Kettering Cancer Center. Protein machinery of vesicular transport.
Stillman, B., Cold Spring Harbor Laboratory. Biochemical approach to understanding the replication of eukaryotic cells.
Early Development of *Xenopus laevis*

April 12–21

INSTRUCTORS

Grainger, Robert, Ph.D., University of Virginia
Sive, Hazel, Ph.D., Whitehead Institute

ASSISTANTS

Doniach, Tabitha, University of California, San Francisco
Gamse, Joshua, Whitehead Institute
Kroll, Kristen, Harvard Medical School

This course provided extensive laboratory exposure to the biology, manipulation, and use of embryos from the frog, *Xenopus laevis*. The course was suited for investigators who have had no experience with *Xenopus* and who have worked with *Xenopus* and wished to learn new techniques. All students had current training in molecular biology and some knowledge of developmental biology. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in both experimental and molecular embryology. Areas covered included (1) care of adults and embryo isolation; (2) stages of embryonic development and anatomy; (3) whole mount in situ hybridization and immunocytochemistry; (4) microinjection of eggs and oocytes, including DNA constructs, mRNA and antisense oligonucleotides; (5) micromanipulation of embryos, including explant and transplantation assays; and (6) use of cell cycle extracts, including preparation of transgenic embryos.

PARTICIPANTS
Ballard, W., Ph.D., CSIRO, Australia
Ben Asher, E., Ph.D., Weizmann Institute of Science, Israel
Gaudierei, S., Ph.D., University of Western Australia
James, C., Ph.D., University of Manchester, United Kingdom
Krall, J., B.S., Amersham Life Sciences, Inc., Cleveland, Ohio
Latinwo, L., Ph.D., Florida A&M University
Lau, C.H., B.S., M.S., National University of Singapore
McPherson, J., Ph.D., University of California, Irvine
Meyers, B., B.A., M.S., University of California, Davis
Myerson, J., B.S., M.S., Merck Research Laboratories

SEMINARS
Grainger, R., University of Virginia, Charlottesville. Xenopus lens induction: From sense to sensibility.
Harland, R., University of California, Berkeley. Expression cloning and the study of Xenopus neural induction.
Heasman, J., University of Minnesota School of Medicine. Adhesion molecules and antisense oligonucleotide inhibition of function.
Keller, R., University of Virginia, Charlottesville. Xenopus gastrulation.

Sive, H., Whitehead Institute, Cambridge, Massachusetts. Anteroposterior ectodermal patterning in Xenopus.
Slack, J., University of Bath, United Kingdom. Fibroblast growth factor signaling during Xenopus development.
Smith, James, National Institute for Medical Research, United Kingdom. Upstream and downstream of brachyury: Xenopus mesoderm induction.
Solomon, Mark, Yale University. Cell cycle extracts and analyzing the Xenopus cell cycle.

Advanced Bacterial Genetics

June 7–27

INSTRUCTORS
Bassler, Bonnie, Ph.D., Princeton University
Manoil, Colin, Ph.D., University of Washington
Trun, Nancy, Ph.D., National Cancer Institute, NIH
The laboratory course presented logic and methods used in the genetic dissection of complex processes in bacteria. The methods presented included mutagenesis using chemical, physical, and biological mutagens; mapping mutations using genetic and physical techniques; analysis of cytoplasmic and membrane proteins using gene fusions; suppressor analysis; molecular cloning; polymerase chain reaction; and epitope insertion mutagenesis. The course consisted of a series of experiments designed to illustrate the genetic logic necessary to begin with a simple phenotype and progress through a detailed characterization of a biological process. A key component of the course was the use of sophisticated genetic methods in the analysis of "undomesticated" bacteria. Invited lecturers described the use of genetic approaches to study processes in a variety of bacteria.

PARTICIPANTS
Canovas, D., B.S., University of Sevilla, Spain
Cook, G.M., M.S., Ph.D., King's College London, United Kingdom
Kamili-Moghaddam, M., M.S., Uppsala University, Sweden
Lee, M.-A., B.S., Ph.D., Defense Medical Research Institute Singapore
Leibler, S., B.A., M.S., Ph.D., Princeton University
Lubys, A., B.S., Institute of Biotechnology, Lithuania
Nelson, D., B.S., Ph.D., University of California, Davis
Newman, D., B.A., Princeton University
Oethinger, M., M.D., Ph.D., Tufts University
Patarakul, K., M.D., Georgetown University Medical Center
Razatos, A., B.S., University of Texas
Rhee, J.H., M.D., Ph.D., Chonnam National University Medical School, Korea
Rinchik, E., Ph.D., Sarah Lawrence College
Venturi, V., Ph.D., International Center for Genetic Engineering & Biotechnology, Italy
Worley, M., B.S., Oregon Health Sciences University
Zhou, Z., B.S., Albert Einstein College of Medicine

SEMINARS
Kaiser, D., Stanford University School of Medicine. Extracellular signaling in Myxococcus.
Miller, V., University of California, Los Angeles. Studying Yersinia enterocolitica virulence factors.
Schuman, H., Columbia University. Genetic analysis of intracellular multiplication by Legionella pneumophila.
Silhavy, T., Princeton University. Genetic analysis of protein interactions in the translocation complex.
Wright, A., Tufts University School of Medicine. Genetic analysis of chromosome segregation in bacteria.
Young, R., Texas A&M University. The problem of bacteriophage lysis: It all comes out in the end!

Molecular Approaches to Ion Channel Biology

June 7–27

INSTRUCTORS
Caldwell, John, Ph.D., University of Colorado
Levinson, Rock, Ph.D., University of Colorado
Maue Robert, Ph.D., Dartmouth Medical School

ASSISTANTS
Koszowski, Adam, University of Colorado
Shah, Ruta, Dartmouth Medical School
This intensive laboratory/lecture course introduced students to the multidisciplinary use of molecular biological, biochemical, immunological, and electrophysiological approaches to the study of ion channels. The laboratory focused on the cellular regulation of channel expression and function. Hands-on exercises included characterization of regulatory elements that control transcription of channel genes, identification of ion channel isoform transcripts and proteins, visualization of channel distributions, and the biophysical analysis of channel isoform function in excitable cells and tissues and in exogenous expression systems. Specific techniques employed included PCR, Western blot, transient transfection and expression, immunocytochemistry, and patch clamp analysis of ionic currents. Lectures covered the techniques employed while providing broad exposure to current issues surrounding ion channel mechanisms and cellular expression. This course was intended for advanced students, postdoctoral researchers, as well as independent investigators with specific plans to apply the techniques taught to a defined problem, and participants were encouraged to bring their preparations to the course for preliminary studies.

PARTICIPANTS
Angsuthanasombat, C., B.S., M.S., Ph.D., Mahidol University, Thailand
Arazi, T., B.S., M.S., Weizmann Institute, Israel
Bautista, D., B.S., Stanford University
Benson, D., B.A., Ph.D., Mount Sinai School of Medicine
Harty, P., B.S., Ph.D., National Institutes of Health
Peter, D., B.S., Ph.D., Rockefeller University
Rose, C., B.S., Ph.D., Yale University School of Medicine
Sierralta, I., M.S., University of Calgary, Canada
Talukder, G., B.S., Stanford University
Tosetti, P., B.S., University of Pavia, Italy
Trevino, C., B.S., Ph.D., Universidad Nacional Autonoma de Mexico
Zhu Y., B.S., Ph.D., New England Medical Center
SEMINARS

Dawson, D., University of Michigan Medical School. The CFTR C1 channel: Molecular basis of conduction and gating.

Goldman, D., University of Michigan. Activity-dependent and synapse-specific expression of nAChR genes during development of the neuromuscular junction.

Green, W., University of Chicago. Assembly of ion channels.

Henderson, L., Dartmouth College. Sex and the single channel: GABA receptors in the rat hypothalamus.

Kusiak, J., National Institutes of Health. Transcriptional control of the NMDAR1 gene.

Mandel, G., State University of New York, Stony Brook. Regulations of neuronal sodium channel genes through transcriptional silencing.

Monyer, H., University of Heidelberg, Germany. Glutamate receptor expression in individual neurons.

Role, L., Columbia University. Distribution and expression of neuronal acetylcholine receptors in the brain.

Sargent, P., University of California, San Francisco. Structural and functional diversity of nicotinic receptor families in the brain.

Snutch, T., University of British Columbia, Canada. Molecular aspects of calcium channel expression in the nervous system.

Trimmer, J., State University of New York, Stony Brook. Protein-protein interactions important in K⁺ channel function.

Molecular Embryology of the Mouse

June 7-27

INSTRUCTORS

Behringer, Richard, Ph.D., University of Texas/M.D. Anderson Cancer Center

Papaioannou, Virginia, Ph.D., Columbia University College of Physicians & Surgeons

CO-INSTRUCTORS

Koopman, Peter, Ph.D., University of Queensland, Australia

Magnuson, Terry, Ph.D., Case Western Reserve University
This course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an intensive introduction into the technical aspects of working with and analyzing mouse embryos, and lecture components provided the conceptual basis for current research. Procedures that were described included isolation and culture of pre- and postimplantation embryos; oviduct and uterus transfer; formation of aggregation chimeras; isolation of germ layers in gastrulation-stage embryos; establishment, culture, and genetic manipulation of embryonic stem cell lines; in situ hybridization to whole mounts of embryos; immunohistochemistry; microinjection of DNA into pronuclei; and microinjection of embryonic stem cell into blastocysts.


SEMINARS

Behringer, R., M.D. Anderson Cancer Center/University of Texas. Organization of pattern during mouse embryogenesis.
Bradley, A., Baylor College of Medicine. Teratocarcinomas and embryonic stem cells. Homologous recombination in mouse embryonic stem cells.
Bronner-Fraser, M., University of California, Irvine. The neural crest.
Hammer, R., University of Texas Southwestern Medical Center. Transgenic animals in biomedical research.
Jessell, T., Columbia University College of Physicians & Surgeons. Patternin of the vertebrate nervous system.
Joyner, A., New York University Medical Center. Mouse engrailed genes and patterning of the brain and limbs.
Koopman, P., University of Queensland, Australia. Sox genes and mouse development.
Lovell-Badge, R., MRC, National Institute for Medical Research, United Kingdom. Mammalian sex determination.
Magnuson, T., Case Western University. Role of eed during mouse gastrulation.
Mann, J., Beckman Research Institute, City of Hope. Genomic imprinting.

McMahon, A., Harvard University. Inductive mechanisms that pattern the mouse embryo.
Nagy, A., Mount Sinai Hospital, Canada. Vasculogenesis in the mouse embryo.
Parada, L., University of Texas Southwestern Medical Center. Role of neurotrophic factors during nervous system development.
Rinchik, E., Sara Lawrence College. Mutagenesis.
Robertson, E., Harvard University. Nodal signaling during gastrulation and axis formation in the mouse.
Rossant, J., Mount Sinai Hospital, Canada. Mouse preimplantation development and implantation. Cell lineage and inductive interactions in the early mouse embryo.
Solter, D., Max-Planck Institute of Immunology, Germany. Genomic imprinting.
Tam, P., Children's Medical Research Institute, Australia. Mouse postimplantation development. Cell lineage and potency in the early mouse embryo.
Wilkinson, D., MRC, National Institute for Medical Research, United Kingdom. Patterning of the vertebrate hindbrain.
This lecture course considered the difficulties in studying the genetic basis of complex disorders, such as diabetes, cardiovascular disease, cancer, Alzheimer's disease, schizophrenia, and epilepsy, with a particular emphasis on neuropsychiatric conditions. Discussions were held on genetic-epidemiologic study designs, including family, twin, and adoption studies, as well as mode of inheritance analyses, and their role in setting the framework for understanding the genetic and nongenetic components of a disease.

A major focus was on the identification of specific gene effects using both linkage and association analysis. Discussed was the efficiency and robustness of different designs for such analysis; further considered was how evidence from genetic-epidemiologic studies informs both the design and interpretation of molecular genetic studies. Recent discoveries of genes for both Mendelian and non-Mendelian diseases guided the discussion of the various methodologic issues.

Neurobiology of Human Neurological Disease:
Mechanisms of Neurodegeneration

INSTRUCTORS

Gandy, Samuel, Ph.D., Cornell University Medical College
Mobley, William, Ph.D., University of California, San Francisco
Prusiner, Stanley, Ph.D., University of California, San Francisco

Why do neurons die in specific acute or chronic human neurological disorders? How do they die? Does apoptosis characterize cell death in some of these disorders? Do different pathological deaths share common mechanisms? What practical treatments can be contemplated?

This lecture course explored possible answers to these important questions. Recent advances in neurogenetics and in molecular and cell biology have begun to shed light on the mechanisms that underlie nervous system injury in disease states such as Alzheimer’s disease, amyotrophic lateral sclerosis, prion diseases, Huntington’s disease, epilepsy, and stroke. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overviews were provided and course participants did not need to have famil-
The course focused principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigation, including the interdependence of clinical research and disease model development, and the value of disease research in understanding the function of the normal nervous system.


PARTICIPANTS
Aguirre, T., B.S., M.S., Katholieke University of Leuven, Belgium
Aleshkov, S., M.D., Ph.D., Boston University Medical Center
Ances, B., B.S., M.S., University of Pennsylvania
Bolwig, G., B.S., Ph.D., Cold Spring Harbor Laboratory
DeMarco, S., B.S., M.S., Mayo Graduate School
Greenfield, J., B.A., Cornell University
Jarrott, B., B.S., Ph.D., Monash University, Australia
Jenkins, S.-A., B.A., University of California, Los Angeles
Lorenzetti, D., B.S., Ph.D., Baylor College of Medicine
Lutjens, R., B.S., Ph.D., Glaxo Institute for Molecular Biology, Switzerland
Meier, J., B.S., Ph.D., McGill University, Canada
Morrison, B., B.A., Mount Sinai Medical School
Neuhaus, R., B.S., Schering AG, Germany
Opal, P., M.D., Ph.D., Northwestern University
Ri, Y., B.S., Albert Einstein College of Medicine
Rogove, A., B.S., State University of New York, Stony Brook
Suopanki, J., B.A., M.S., University of Helsinki, Finland
Vali, S., B.S., University of California, Davis
Vassar, R., B.A., Ph.D., AMgen, Inc.
Wyoral, S., B.S., Columbia University College of Physicians & Surgeons
Xu, R., B.S., M.S., AMgen, Inc.

SEMINARS
Bredesen, D., La Jolla Cancer Research Foundation.
Thanatopsis: Principles emerging from the study of neural apoptosis.
Choi, D., Washington University School of Medicine.
Huntington’s patient. Excitotoxicity.
Dawson, V., Johns Hopkins University School of Medicine.
Free radicals and oxidants: Mechanisms and pathways to neurodegeneration.
Edwards, R., University of California, San Francisco.
Parkinson’s disease.
Gandy, S., Cornell University Medical College.
Regulation of amyloid metabolism.
Heintz, N., Rockefeller University.
Ataxia telangiectasia: Cell cycle checkpoints and neurodegeneration.
McNamara, J., Duke University Medical Center.
Emerging insights into mechanisms of epilepsy.
Mobley, W., University of California, San Francisco.
Neurotrophic factors and neurologic disease. AD patient.
Prusiner, S., University of California, San Francisco.
Prion diseases.
Ross, C., Johns Hopkins University School of Medicine.
Triplet repeat neurodegenerative disorders: Focus on Huntington’s disease.
Tanzi, R., Massachusetts General Hospital.
Molecular analysis of the four genes responsible for familial Alzheimer’s disease.
Selkoe, D., Harvard Medical School.
Molecular pathogenesis of Alzheimer’s disease.
Sisodia, S., Johns Hopkins University School of Medicine.
Animal models of AD.
Computational Neuroscience: Vision

June 28–July 11

INSTRUCTORS
Heeger, David, Ph.D., Stanford University
Shadlen, Michael, Ph.D., University of Washington, Seattle
Simoncelli, Eero, Ph.D., University of Pennsylvania

ASSISTANTS
Boynton, Geoffrey, Stanford University
Teo, Patrick, Stanford University

Computational approaches to neuroscience have produced important advances in our understanding of neural processing. Prominent successes have come in areas where strong inputs from neurobiological, behavioral and computational approaches can interact. Through a combination of lectures and hands-on experience with a computer laboratory, this intensive course examined several areas, including feature extraction, motion analysis, binocular stereopsis, color vision, higher-level visual processing, visual neural networks, and oculomotor function. The theme was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience. Students had experience in neurobiological or computational approaches to visual processing. Some background in mathematics was beneficial.

This course provided an intensive overview of topics in plant growth and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from Arabidopsis thaliana and other model plants and provides an introduction to current methods used in Arabidopsis research. The course also demonstrated the use of microbial systems in plant research, including Agrobacterium, E. coli, and S. cerevisiae. It was designed for scientists with ex-
perience in molecular techniques or in plant biology who wish to work with Arabidopsis. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Speakers provided both an in-depth discussion of their own work and a review of their specialty.

Discussions of important topics in plant research were presented by the instructors and by invited speakers. These seminars reviewed plant anatomy; plant development (including development of flowers, roots, meristems, embryos, and the epidermis); perception of light and photomorphogenesis; responses to pathogens and to other environmental stresses; synthesis and function of secondary metabolites and hormones; nitrogen assimilation; unique aspects of plant cell biology (including the plant cytoskeleton, cell wall, and chloroplasts); the importance of transposons and Agrobacterium for manipulating plant genomes; and current approaches to genome analysis.

The laboratory sessions provided an introduction to important techniques currently used in Arabidopsis research. These included studies of Arabidopsis development, mutant analysis, studies of epidermal features, in situ detection of RNA, histochemical staining and immunolabeling of proteins, transformation with Agrobacterium, transient gene expression in protoplasts, expression of plant proteins in microorganisms, detection and analysis of plant pathogens, and techniques commonly used in genetic and physical mapping.


PARTICIPANTS
Bueche, C., B.S., Institut fur Biologie, II, Germany
Cairney, J., B.S., Ph.D., Institute of Paper Science & Technology, Atlanta, Georgia.
Carabelli, M., Ph.D., Centro Acidi Nucleici, CNR, Italy
Gosti, F., B.S., Ph.D., National Research Science Center, France
Kent, M., B.S., University of Minnesota
Kodrzycki, R., B.S., Ph.D., Westvaco Corporation, South Carolina
Larrinua, I., B.S., Ph.D., Dowelanco Discovery Research, Indiana
Matthews, Jr., W., B.S., M.S., University of California, Riverside
Queitsch, C., B.S., University of Chicago
Raventos, D., B.S., Ph.D., University of Copenhagen, Denmark
Venkataraman, S., B.S., Michigan State University
Weber, A., B.S., M.S., Harvard University
Yalpani, N., B.S., Ph.D., Pioneer Hi-Bred International, Inc., Iowa
Zeidler, M., B.S., M.S., Fu Berlin, Germany
Zik, M., B.S., M.S., Weizmann Institute of Science
This laboratory/lecture course was intended for researchers at all levels who want to use Drosophila as an experimental system for studying behavior, physiology, and development. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and/or direct laboratory exercises and experiments in their areas of interest.

The course provided students in hands-on experience with various wild-type and mutant preparations useful for studying behavioral and neurobiological questions: Larval and adult nervous systems are used for studying behavior and physiology; embryonic and metamorphosing nervous systems are used for studying development. Students were exposed to a variety of techniques, such as genetic manipulations for generating and managing stocks, chromosomal in situ hybridization, and embryo injections, providing a comprehensive introduction to modern experimental tools specific for Drosophila.

In last year’s course, behavioral emphasis was given to courtship, learning and memory, and biological rhythms; physiological emphasis was given to the genetics and molecular biology of excitability; and developmental emphasis was given to neurogenesis, axonal pathfinding, and synaptogenesis.

PARTICIPANTS
Andretic R., B.S., University of Virginia
Chouard, T., B.S., M.S., Ph.D., University of California, Los Angeles
Gray, C., B.A., University of Virginia
Hammerle, B., B.S., Ph.D., Instituto Cajal, CSIC, Spain
Hoang B., B.S., University of Illinois
Hodge, J., B.S., Cambridge University, United Kingdom
Thomas M., B.S., M.S., University of Zurich, Switzerland
Mukhopadhyay, M., B.S., M.S., MacMaster University, Canada
Schuldt, A.J., B.S., Cambridge University, United Kingdom
Wenniger, J., B.A., University of Pennsylvania
Wu, M., B.A., Baylor College of Medicine
Yu, H.-H., B.A, M.S., Johns Hopkins University School of Medicine

SEMINARS
Brand, A., Wellcome/CRC Institute, United Kingdom.
  Determination and function of glia/midline cells.
Carlson, J., Yale University. Olfactory processing.
Ganetzky, B., University of Wisconsin. Introductory physiology, ion channels.
Hartenstein, V., University of California, Los Angeles. Embryonic development.
Jackson, R., Worcester Foundation for Biomedical Research. Circadian behaviors.
Kernan, M., State University of New York, Stony Brook. Mechanosensory systems.
Kolodkin, A., Johns Hopkins School of Medicine. Axon pathfinding.
Montell, C., Johns Hopkins School of Medicine. Visual systems.
Murphey, R., University of Massachusetts. Sensory systems and adult behaviors.
Patel, N., University of Chicago. Pattern formation/neurogenesis/evolution.
Sink, H., University of California, Berkeley. Development of motor systems.
Restifo, L., University of Arizona. Metamorphosis and adult development.
Sokolowski, M., York University, Canada. Larval behaviors.
Taghert, P., Washington University School of Medicine.
  Neuromodulators and neurotransmitters.
Taylor, B., Oregon State University. Reproductive behaviors and the adult nervous system.
Tully, T., Cold Spring Harbor Laboratory. Learning and memory.
Wu, C.-F., University of Iowa. Physiology of motor systems.
Molecular Cloning of Neural Genes

July 1–21

INSTRUCTORS
Boultar, James, Ph.D., University of California, Los Angeles
Darnell, Robert, Ph.D., Rockefeller University
Heintz, Nathaniel, Ph.D., Rockefeller University
Lai, Cary, Ph.D., Scripps Research Institute

CO-INSTRUCTORS
Elgoyhen, Ana Belen, Salk Institute
Kong, Hae-Young, University of Pennsylvania
Quinn, Anne Marie, Yale University

ASSISTANTS
Arnold, Donald, Rockefeller University
Okano, James, Rockefeller University
Zeltsar, Lori, Rockefeller University

This intensive laboratory and lecture course was intended to provide neuroscientists at all levels with an introduction to modern molecular neurobiology. The course consisted of daily laboratory exercises, discussions on the practice of molecular biology, and a series of evening research seminars by invited speakers. This lecture series emphasized the ways in which molecular techniques studied in the laboratory have been successfully applied to the study of neural genes.

The laboratory portion of the course included a module on gene cloning and a module on the analysis of neural gene expression. Gene cloning techniques included isolation and characteriza-
tion of poly(A)⁺ RNA; synthesis of cDNA libraries; standard screening and expression screening of cDNA libraries; analysis of cDNA clones, including phagemid rescue, restriction analysis, Southern blotting, ligations, transformation, electroporation, and subcloning; and PCR analysis including oligonucleotide design, synthesis, and purification, and PCR-based subtraction cloning. Gene expression studies included Northern blot analysis, RT-PCR analysis, in vitro transcription for RNase protection, and in situ and mammalian gene targeting.


PARTICIPANTS
Canzoniero, L., M.D., Ph.D., Washington University
Conn, P.J., B.S, Ph.D., Emory University
Dougherty, M., B.S., Ph.D., University Pierre & Marie Curie, France
Hajihosseini, M., B.S., M.S., Ph.D., Pasteur Institute, France
Hall, J., B.S., Cambridge University, United Kingdom
Horton, A., B.S., University of St. Andrews, United Kingdom
Jasmin, L., M.D., Ph.D., Georgetown University Medical Center
Jiang, H., B.S., M.S., Purdue University
Koentges, G., B.S, UMDS, Guy's & St. Thomas's Medical & Dental Schools, United Kingdom
Korade, Z., DVM., Ph.D., University of Pittsburgh
Korotzer, A., B.A., Ph.D., Colorado State University
Lander, C., B.A., Yale University
McKinnon, S., B.S., M.S., Ph.D., Johns Hopkins University School of Medicine
Nicholson, L., B.S., M.S., Ph.D., Salk Institute, United Kingdom
Rosenmund, C., B.S., Ph.D., Max-Planck Institute, Germany
Yoon, C., B.A., Harvard Medical School

SEMINARS
Chao, M., Cornell University Medical School. Molecular cloning of neural genes.
DeCamilli, P., Yale University School of Medicine. Molecular mechanisms in synaptic vesicle endocytosis.
Eberwine, J., University of Pennsylvania. RNA-binding proteins and neurologic disease.
Friedman, J., Rockefeller University. Genetic analysis of neural disease genes.
Harlow, E., Massachusetts General Hospital Cancer Center. Approaches to gene cloning in the brain.

Neurobiology: Brain Development and Function

July 14–27

INSTRUCTORS
McKay, Ronald, Ph.D., National Institutes of Health
Schuman, Erin, Ph.D., California Institute of Technology

This lecture course presented both basic concepts and currently exciting research problems in neurobiology. It focused on the methods now used to study the development and function of the nervous system. Topics covered included gene expression, cell proliferation and tumor formation,
neural induction, stem cells and lineage, neurite outgrowth, synaptic biochemistry and physiology, positional information and maps, learning, sensory transduction and coding, transplantation, transgenic mice, and behavior. The advantages of different experimental systems from *C. elegans* to primates were discussed. The main purpose of this course was to provide many opportunities to discuss this rapidly expanding field with invited lecturers. The lectures provided a general introduction as well as a detailed analysis of current research for each topic. Individuals from a wide variety of backgrounds and experience were encouraged to apply.


PARTICIPANTS
Attix, S., B.S., University of California, Los Angeles
Bhattacharya, S., B.A., Ph.D., Stanford University
Blanquet, V., B.S., Ph.D., GSF-Forschungszentrum, Germany
DiPaolo, G., B.S., M.S., Glaxo Institute for Molecular Biology, Switzerland
Gao, F.B., B.S., Ph.D., University College London, United Kingdom
Gotoh, Y., B.S., M.S., Ph.D., Kyoto University, Japan
Grishchenko, E., B.S., Kiev National University, Ukraine
Gulisano, M., M.D., Ph.D., Scientific Institute H. San Raffaele, Italy
Hargrave, M., B.S., University of Queensland, Australia
Hu, H., B.S., Ph.D., Case Western Reserve University
Jellie, A., B.S., M.S., Ph.D., University of Otago, New Zealand
Johansen, J., B.S., M.S., Karolinska Institute, Sweden
Johansson, C., B.S., D.D.S., Karolinska Institute, Sweden
Joore, J., B.S., M.S., Ph.D., Netherlands Institute for Developmental Biology, The Netherlands
Kohwi, Y., B.S., Ph.D., Burnham Institute
Krug, K., B.A., University Laboratory of Physiology, United Kingdom
Margolis, R., B.A., M.D., Johns Hopkins University
Neophytou, C., B.A., MRC, University College London, United Kingdom
Pizzorusso, T., B.S., Ph.D., Scuola Normale Superiore, Italy
Price, S., B.A., Medical Research Council, United Kingdom
Salecker, I., B.S., Ph.D., University of California, Los Angeles
Eukaryotic Gene Expression

July 24–August 13

INSTRUCTORS

Kenneth Burtis, Ph.D., University of California, Davis
Michael Carey, Ph.D., University of California, Los Angeles
Stephen Smale, Ph.D., University of California, Los Angeles

ASSISTANTS

Kate Ellwood, University of California, Los Angeles
Christopher Kaelin, University of California, Davis
Le Trinh, University of California, Los Angeles

This course focused on the techniques used to study eukaryotic gene expression, particularly with respect to transcriptional regulation. We examined the role of both cis- and trans-acting components in this process. Regulation of gene expression by nuclear proteins was examined both by in vitro transcription assays using cell-free extracts and by transfection of cloned DNA into mammalian tissue culture cells. Analytical techniques for these expression studies included primer extension, nuclease protection, and enzymatic assays for reporter proteins. Eukaryotic transcription factors were expressed in E. coli and purified by affinity chromatography. Using these proteins, students learned the techniques for detecting and characterizing the interaction between regulatory DNA sequences and trans-acting protein factors, including mobility shift assays, DNase footprinting, and methylation interference. Students also learned techniques for carrying out site-directed mutagenesis of the sequences encoding trans-acting regulatory proteins as well as cis-acting upstream regulatory elements. The two-hybrid technique in S. cerevisiae was employed to examine protein-protein interactions involved in transcriptional activation. Experience with basic recombinant DNA techniques is a prerequisite for admission to this course.
Lectures by the instructors covered the theoretical aspects of the techniques used as well as broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes. Guest lecturers discussed current problems in eukaryotic molecular biology as well as technical approaches to their solution. Speakers included S. Bell, S. Burley, J. Conaway, S. Ghosh, D. Gilmour, B. Graves, M. Green, R. Sen, B. Stillman, K. Struhl, and K. Yamamoto.

PARTICIPANTS
Bauer, A., B.S., I.M.P., Research Institute of Molecular Pathology, Austria
Blemings, K., B.S., Ph.D., University of Wisconsin
Burczynski, M., B.S., University of Pennsylvania School of Medicine
Garcia, J., B.S., M.S., Centro de Investigacion y de Estudios Avanzados del Instituto Politecnico Nacional, Mexico
Gaudet, P., B.S., Concordia University, Canada
Heller, R., B.S., M.D., University of Oxford, United Kingdom
Hurle, B., B.S., Universidad de Oviedo, Spain
Kamnert, I., B.S., University of Lund, Sweden

SEMINARS
Bell, S., Massachusetts Institute of Technology. Biochemical characterization of the ORC proteins.
Burley, S., Rockefeller University. X-ray crystallographic studies of eukaryotic transcription factors.
Burtis, K., University of California, Davis. Structure and function of the doublesex proteins of D. melanogaster.
Carey, M., University of California, Los Angeles. Biochemical mechanisms of eukaryotic gene activation.
Conway, J., Oklahoma Medical Research Foundation. Factors and mechanisms controlling elongation by RNA polymerase II.
Freedman, L., Memorial Sloan-Kettering Institute. Transcriptional activation and repression by nuclear hormone receptors.
Ghosh, S., Yale Medical School. Differential regulation of NF-κB by I-κBa and I-κBβ.

Karpenko, Oxano, B.S., Brown University
Kurie, J., B.A., M.D., M.D. Anderson Cancer Center/University of Texas
Li, L., D.V.M., Ph.D., Rockefeller University
Penc, S., B.S., M.S., Ph.D., Harvard Medical School
Ronnov-Jessen, L., B.S., Ph.D., Rigshospitalet, Denmark
Samaras, S., B.S., Ph.D., Pennsylvania State University
Sokolowski, M., B.S., Karolinska Institute, Sweden
Ten Berge, D., B.S., M.S., The Netherlands Institute of Developmental Biology, The Netherlands

Graves, B., University of Utah. The Ets family of transcription factors: Winged helix-turn-helix proteins displaying autoinhibition of DNA binding.
Green, M., University of Massachusetts. Analysis of TAFII function in vivo.
Peterson, C., University of Massachusetts. Protein complexes for remodeling chromatin.
Sen, R., Brandeis University. Factors regulating Ig gene expression.
Smale, S., University of California, Los Angeles. Regulation of TATA-less genes in immature lymphocytes.
Stillman, B., Cold Spring Harbor Laboratory. Cell cycle control of genome replication.
Tanese, N., New York University. Transcriptional activation mediated by the mammalian TFIID complex.
Yamamoto, K., University of California, San Francisco. Signaling and transcriptional regulation by intracellular receptors.
Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for visualizing and measuring the structure and function of neurons, synapses, and networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical tools to utilize these emerging technologies. The primary emphasis of the course was on light microscopy, including fluorescence, differential interference contrast, confocal scanning, and 2-photon scanning excitation, as well as the use of different types of electronic cameras and the application of digital processing to enhance and analyze microscope images. Students learned the principles of light microscopy, the use of calcium-sensitive probes (e.g., Fura-2, calcium green, and oregon BAPTA green), voltage-sensitive dyes, photo-activated ("caged") compounds, GFP, exocytosis tracers, whole-cell patch-clamp methods in brain slices, single-cell microinjection of fluorescent indicators, and other methods to explore the molecular determinants of neuronal function. The course used a variety of neural systems, including living animals, brain slices (e.g., hippocampus, cerebellum, and neocortex), and cultured cells. Applicants had a strong background in the neurosciences, physiology, or cell biology.

Yeast Genetics

July 24–August 13

INSTRUCTORS
Adams, Alison, Ph.D., University of Arizona
Gottschling, Daniel, Ph.D., Fred Hutchinson Cancer Research Center
Kaiser, Chris, Ph.D., Massachusetts Institute of Technology

CO-INSTRUCTOR
Segev, Nava, University of Chicago

ASSISTANTS
Chen, Esther, Massachusetts Institute of Chicago
Meisinger, Lia, University of Chicago
O’Dell, Johanna, University of Arizona

The major laboratory techniques used in the genetic analysis of yeast were studied, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombinat-
tion. Micromanipulation used in tetrad analysis were carried out by all students. Molecular genetic
techniques, including yeast transformation, gene replacement, analysis of gene fusions, and gen-
eration of mutations in cloned genes, were studied. Indirect immunofluorescence experiments were
done to identify the nucleus, microtubules, and other cellular components. Lectures that are funda-
mental aspects of yeast genetics were presented along with seminars given by outside speakers
on topics of current interest.

PARTICIPANTS
Adams, C., B.S., Ph.D., University of Bath, United Kingdom
Choi, J.H., B.S., M.S., St. John’s University
Clarke, E., B.S., University of Oklahoma
Cox, G., B.A., M.D., Duke University Medical Center
Eisenstein, R., B.A., Ph.D., University of Wisconsin
Fisher, E., B.A., M.D., Ph.D., Mount Sinai School of Medicine
Hatsuzawa, K., B.S., Ph.D., Tokyo University of Pharmaceuti-
cal and Life Sciences, Japan
Lin, J., B.S., Ph.D., Cornell University
Liu, W.-M., B.A., M.S., Ph.D., Stanford University
Meinke, M., B.S., Ph.D., St. John’s University/College of St.
Benedict, Minnesota
Mizuguchi, M., B.S., M.S., Ph.D., National Institutes of Health
Schultz, M., B.S., Ph.D., University of Alberta, Canada
Slayman, C., B.A., Ph.D., Yale University School of Medicine
Smits, G., B.S., University of Amsterdam, The Netherlands
Takagi, H., B.S., M.S., Ph.D., Fukui Prefectural University,
Japan
Tinker-Kulberg, R., B.S., Ph.D., University of California, San
Francisco

SEMINARS
Adams, A., University of Arizona. The actin cytoskeleton of
yeast.
Botstein, D., Stanford University School of Medicine. Yeast
genetics in the postgenome era.
Fink, G., Whitehead Institute. Signal transduction and dimor-
phism.
Futcher, B., Cold Spring Harbor Laboratory. The yeast cell
cycle engine.
Gottschling, D., Fred Hutchinson Cancer Research Center.
Telomeric position effect: A handle on the chromosome.
Herskowitz, I., University of California, San Francisco. Cell
specialization and the life cycle of yeast.
Hieter, P., Johns Hopkins University. Chromosome segrega-
tion.
Kaiser, C., Massachusetts Institute of Technology. Regula-
tion of vesicle transport in the secretory pathway.
Mitchell, A., Columbia University. Regulation of entry into
meiosis.
Parker, R., University of Arizona. mRNA turnover in yeast.
Peterson, C., University of Massachusetts Medical Center.
Molecular machines for remodeling chromatin.
Petes, T., University of North Carolina. Genetic control of
genome stability in yeast.
Rose, M., Princeton University. Karyogamy and spindle pole
body function.
Segev, N., University of Chicago. GTPases in protein trans-
port.
Struhl, K., Harvard Medical School. Regulation of transcrip-
tion.
Wickner, R., National Institutes of Health. Yeast killer and
regulation of protein states.
Advanced Drosophila Genetics

July 30–August 12

INSTRUCTORS
Ashburner, Michael, Ph.D., University of Cambridge, United Kingdom
Hawley, Scott, Ph.D., University of California, Davis

This intensive seminar course provided an introduction to the theory and practice of methods used to manipulate the Drosophila genome. It was suitable for graduate students and researchers with some experience with Drosophila who were interested in expanding their knowledge of the wide range of genetic techniques now available for use with this organism. Topics covered included chromosome mechanics, the design and execution of genetic screens, and the use of transposable elements as genetic tools.

PARTICIPANTS
Adler, E., B.A., Ph.D., Institut de Biologie Physico-Chimique, France
Bartsch, S., B.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Breuer, S., B.S., Philipps-Universitat, Germany
Franc, N.C., B.S., CNRS, Institut de Biologie Moleculaire et Cellulaire, France
DeYoung, K., B.S., National Institutes of Health
Dubois, L., B.S., Universite Paul Sabatier Toulouse, France
Ekengren, S., B.S., M.S., Stockholm University, Sweden
Faye, I., B.S., Ph.D., Stockholm University, Sweden
Goldstein, D., B.S., Ph.D., Pennsylvania State University
Ip, T., B.S., Ph.D., University of Massachusetts Medical Center
Khalsa, O., B.A., Brown University
Luk, S.K.-S., B.S., M.S., Ph.D., Chinese University of Hong Kong
Maggert, K., B.S., University of California, San Diego and the Salk Institute
Neal, K., B.A., Ph.D., Emory University
Yan, R., B.S., Ph.D., Rockefeller University
Pearson, A., B.A., Massachusetts Institute of Technology
Torres, S., B.S., Ph.D., Brown University
Walker, D., B.A., Iowa State University
Zwick, M., B.S., University of California, Davis
SEMINARS
Ashburner, M., University of Cambridge, United Kingdom. Introduction to Drosophila biology, phylogeny.
FlyBase—where to find information. Chromosomes, cytogenetics, chromosome aberrations, mapping (genetic and cytogenetic).
Cherbas, P., Indiana University. Somatic cell genetics.
Engels, W., University of Wisconsin. P elements.
Golic, K., University of Utah. Mosaic systems FRT/FLP.
Hawley, S., University of California, Davis. Genetics of meiosis; methods of study and exploitation; distributive pairing.
Hogness, D., Stanford University School of Medicine. The Drosophila genome: Past, present, and future.
Lehmann, R., New York University Medical Center/Skirball Institute. Genetic analysis of early Drosophila development.
Simpson, P., I.G.B.M.C., France. The achaete-scute complex and the genetic control of bristle pattern.
Theurkauf, W., State University of New York, Stony Brook. Genetic analysis of cytoskeletal function during oogenesis and early embryogenesis.
Tully, T., Cold Spring Harbor Laboratory. Genetic dissection of memory.

Advanced In Situ Hybridization and Immunocytochemistry

October 10–23

INSTRUCTORS
Hough, Paul, Ph.D., Brookhaven National Laboratory
Jacobson, Kenneth, Ph.D., University of North Carolina, Chapel Hill
Mastrangelo, Iris, Ph.D., Brookhaven National Laboratory
Ried, Thomas, Ph.D., National Institutes of Health
Spector, David, Ph.D., Cold Spring Harbor Laboratory

ASSISTANTS
Howard, Tamara, Cold Spring Harbor Laboratory
Navarre, Richard, University of North Carolina, Chapel Hill
Veldman, Timothy, National Institutes of Health

This course focused on specialized techniques in microscopy related to localizing DNA sequences and proteins in cells and preparing DNA and DNA-protein spreads for microscopic examination. The course emphasized the use of the latest equipment and techniques in epifluorescence microscopy, confocal laser scanning microscopy, electron microscopy, and digital image processing. The aims of the course were designed to provide state-of-the-art technology and scientific expertise in the use of microscopic applications to address basic questions in genome organization and cellular and molecular biology. The course was designed for the molecular biologist who is in need of microscopic approaches and for the cell biologist who is not familiar with the practical application of the advanced techniques presented in the course. Among the methods presented was the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, spectral karyotyping, use of a variety of reporter molecules and nonantibody fluorescent tags, and indirect antibody labeling of multiple proteins in a single cell. In addition, molecular electron microscopy was used to examine DNA-protein interactions. In each method, several experimental protocols were presented allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring nucleic acid or protein probes to the course which were used in addition to those provided by the instructors. The laboratory portion of the course was supplemented by invited lecturers who gave up-to-the-minute reports on current research using the techniques presented in the course.
PARTICIPANTS
Brisken, C., M.D., Whitehead Institute, Massachusetts
Chevret, E., B.S., M.S., Ph.D., Imperial Cancer Research Fund, United Kingdom
Cone, R., M.D., Ph.D., University Hospital Zurich, Switzerland
Dow, J., M.D., Meharry Medical College, Tennessee
Fiorczyk, M., B.S., State University of New York, Stony Brook
Galetta, G., B.A., Ph.D., University of California, San Diego
Horst, H., M.D., Ph.D., University Ulm, Germany
Juan, V., B.A., University of California, Santa Cruz

SEMINARS
Brinkley, W., Baylor College of Medicine. DNA/protein organization in the centromere-kinetochore complex of human chromosomes. An integrated microscopy study.
Cremer, T., University of Heidelberg, Germany. Three-dimensional organization of chromosome territories in the interphase nucleus.
Deerinck, T., University of California, San Diego. Electron microscopy as a tool in cell and molecular biology.
Jacobson, K., University of North Carolina. Basic introduction to light microscopy and video microscopy. Fluorescence microscopy and low light level cameras.

Liva, S., B.S., University of California, Los Angeles
Mermoud, J., B.S., Ph.D., Yale University School of Medicine
Novakovic, S., M.S., M.D., Roche Bioscience, California
Reinert, B., B.S., M.S., Kansas State University
Tsai, C.-C., D.V.M., Ph.D., University of Washington, Seattle
Van Den Berghe, J., B.A., Ph.D., National University of Singapore
Wang, G., B.S., Ph.D., Loma Linda University, California
Wimpee, B., B.S., University of Wisconsin, Milwaukee

Ried, T., National Institutes of Health. Comparative genomic hybridization.
Singer, R., University of Massachusetts Medical School. Cytoplasmic organization of mRNA.
Spector, D., Cold Spring Harbor Laboratory. Immunocytochemistry. An integrated microscopic approach to examining nuclear organization.
Macromolecular Crystallography

October 10–23

INSTRUCTORS
Furey, William, Ph.D., V.A. Medical Center
Gilliland, Gary, Ph.D., Center for Advanced Research in Biotechnology
McPherson, Alexander, Ph.D., University of California, Riverside
Pflugrath, James, Ph.D., Molecular Structure Corporation

ASSISTANT
Vasquez, Gregory, Center for Advanced Research in Biotechnology

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This "intensive" laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, crystal freezing, data collection, data reduction, anomalous dispersion, multiple isomorphous replacement, phase determination, solvent flattening, molecular replacement and averaging, electron density interpretation, structure refinement, molecular graphics, molecular dynamics, and multidimensional NMR. Participants learned through extensive hands-on experiments how to crystallize and determine a protein structure, along with lectures and informal discussions on the theory behind the techniques.
PARTICIPANTS
Adler, M., B.A., M.S., Ph.D., Berlex Biosience, California
Dutta, S., B.S., M.S., Boston University
Hanlon, N., B.S., Ph.D., University of Oxford, United Kingdom
Jovine, L., B.S., MRC Laboratory of Molecular Biology, United Kingdom
Kulathila, R., B.S., Ciba-Geigy Corporation, New Jersey
Le, H., B.S., Ph.D., Schering-Plough Research Institute, New Jersey
MacDonald, R., B.A., Ph.D., Northwestern University, Illinois
Monem, V., B.S., Ontario Cancer Institute, Canada
Persson, R., B.S., Lund University, Sweden

SEMINARS
Brunger, A., Yale University, HHMI. Accurate experimental crystallographic phases by multiwavelength anomalous dispersion.
Clore, G.M., National Institutes of Health. Structures of protein-DNA complexes by NMR.
Doudna, J., Yale University. A high-resolution tour of a large ribozyme domain.
Dyda, F., National Institutes of Health. Structure determination of HIV integrase core domain by combined MAD.
Gilliland, G., Center for Advanced Research in Biotechnology, Rockville, Maryland. Glutathione S-transferase: Insight into catalysis, substrate specificity, and subunit interactions from structural analyses.
Hendrickson, W., Columbia University, HHMI. Structural biology of cellular responses to stress.
Joshua-Tor, L., Cold Spring Harbor Laboratory. The yeast bleomycin hydrolase, Ga16—A nucleic-acid-binding protease.
Sussman, J., Brookhaven National Laboratory, Upton, New York. Acetylcholinesterase in 3D: New mysteries revealed from the crystal structure.
Tronrud, D., University of Oregon. Strange density.

YACs in Structural and Biological Genome Analysis

October 10–23

INSTRUCTORS
Huxley, Clare, Ph.D., Imperial College School of Medicine at St. Mary’s, United Kingdom
Reeves, Roger, Ph.D., Johns Hopkins University

COINSTRUCTOR
Church, Deanna, Ph.D., University of California, Irvine

ASSISTANTS
Cabin, Deborah, Johns Hopkins University
Clines, Greg, University of Texas Southwestern Medical Center
Mann, Katherine, Imperial College School of Medicine/St. Mary’s Hospital, United Kingdom

This lab-based course was aimed at investigators using genetic and physical mapping to isolate their gene of interest. It covered techniques involved in physical mapping, contig building, gene isolation, and functional analysis of cloned DNA. Experiments included analysis and characterization of large-insert clones (YACs, BACs, and PACs) by partial digestion and pulsed-field gel electrophoresis; creation of nested derivatives of YACs by fragmentation at repeat sequences or exons; end sequence rescue; contig building by STS content mapping; gene identification by cDNA selection and exon trapping; manipulation of YAC DNA by homologous recombination for the intro-
duction of mammalian selectable markers and specific mutations; and preparation of YAC DNA for transfer into cells in tissue culture by lipofection and into transgenic mice by pronuclear injection. There also were computer-based sessions on accessing data from the World Wide Web and using databases. The practical component of the course was supplemented with lectures by invited speakers who are prominent researchers in the field and who then spent time talking to the participants. Participants presented their own research interests to serve as the basis for structured discussions of how to apply current technologies to their specific research project.

PARTICIPANTS
Bartsch, D., B.S., Ph.D., Columbia University
Bullejos, M., B.S., Universidad de Granada, Spain
Galli, J., M.S., Ph.D., Karolinska Hospital, Sweden
Immervoll, T., B.S., Ph.D., GSF Research Center, Germany
Kokkinaki, M., B.S., University of Crete, Greece
Langford, G., B.S., Ph.D., Cambridge University, United Kingdom
Lorenzi, H., B.S., Universidad de Buenos Aires, Argentina
Messiaen, L., B.S., M.S., Ph.D., University Hospital, Belgium
Meyer, A., B.S., Ph.D., Sandoz Pharma Ltd., Switzerland
Monyer, H., B.S., Ph.D., University of Heidelberg, Germany
Parmer, R., M.D., Ph.D., University of California, San Diego
Puranam, R., B.S., M.S., Ph.D., Duke University
Shiraki, T., B.A., M.S., Kyoto University, Japan
Takacs, L., M.D., Ph.D., AMgen, California
Williams, J., M.S., Ph.D., Louisiana State University Medical Center
Yuan, B.-Z., M.D., Ph.D., National Institutes of Health

SEMINARS
Birren, B., Whitehead Institute/MIT Center For Genome Research. Bacterial artificial chromosomes in genome analysis.
Cabin, D., Johns Hopkins University School of Medicine. Fundamentals of yeast genetics for genome analysis.
Clines, G., University of Texas Southwestern Medical Center, Dallas. Gene identification by cDNA selection.
Church, D., University of California, Irvine. Molecular analysis of the Cri du Chat region.
Green, E., National Institutes of Health, NCHGR. YAC-based mapping of human chromosomes.
Friedman, J., Rockefeller University. Positional cloning of obesity genes—The morning after.
Huxley, C., Imperial College, United Kingdom. Functional analysis of mammalian DNA function by YAC transfer.
Reeves, R., Johns Hopkins University. From comparative maps to comparative source.
Trask, B., University of Washington, Seattle. Genome analysis through fluorescence in situ hybridization.
Wilson, R., Washington University School of Medicine, St. Louis, Missouri. Genome analysis by large-scale DNA sequencing.
Mouse Behavioral Analysis

October 25–November 7

INSTRUCTORS
Eichenbaum, Howard, Ph.D., Boston University
Morris, Richard, Ph.D., University of Edinburgh, United Kingdom
Silva, Alcino, Ph.D., Cold Spring Harbor Laboratory

ASSISTANT
Bourtchuladze Roussoudan, Ph.D., Columbia University
Kogan, Jeff, Cold Spring Harbor Laboratory
Wolfer, David, University of Zurich, Switzerland

This course was intended to provide a theoretical and experimental introduction to behavioral analysis in the mouse, with a focus on learning and memory. It was especially designed for geneticists, molecular biologists, pharmacologists, and electrophysiologists with a need for hands-on introduction to behavioral analysis of the mouse. Additionally, the course covered the principles of using mutant mice in behavioral studies, as well as the issues involved in integrating behavioral, neuroanatomical, neurophysiological, and molecular findings. Among the methods presented were the water maze, cued and contextual fear conditioning, inhibitory avoidance, natural/ethologically relevant learning, open field behavior, and other activity tests. In addition, there were demonstrations of several aspects in in vivo and in vitro electrophysiology (synaptic, single unit, and EEG recordings).
PARTICIPANTS
Episkopou, V., B.S., Ph.D., MRC Clinical Sciences Center, United Kingdom
Freeman, T., B.S., University of Oxford, United Kingdom
Gass, P., M.D., Ph.D., German Cancer Research Center
Gianfranceschi, L., B.S., Scuola Normale Superiore, Italy
Johnson, D., M.S., Ph.D., Oak Ridge National Laboratory, Tennessee
Kuhn, R., B.S., Ph.D., University of Cologne, Germany

SEMINARS
Diamond, A., Kennedy Shriver Center, Waltham, Massachusetts. Human cognition—What is the value of mouse models?
Jacobs, L., University of California, Berkeley. The natural mouse.
Jaffard, R., C.N.R.S., URA 339, Talance, France. The hippocampus—What is it good for?
Keverne, B., University of Cambridge, United Kingdom. Beyond learning: What other behavioral issues could be studied in the mouse?
Malinow, R., Cold Spring Harbor Laboratory. Mechanisms of hippocampal synaptic plasticity.
Paylor, R., National Institutes of Health. Neurological tests in mice.
Tonegawa, S., Massachusetts Institute of Technology. Gene targeting and the study of learning and memory.
Wilson, M., Massachusetts Institute of Technology. Critical issues in the integration of behavioral and electrophysiological studies.

Computational Genomics
October 31–November 5

INSTRUCTORS
Pearson, William, Ph.D., University of Virginia, Charlottesville
Smith, Randall, Ph.D., SmithKline Beecham Pharmaceuticals
This course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches to extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, multiple sequence alignment, and hidden Markov models (HMMs). Additional topics included gene recognition (exon/intron prediction), identifying signals in unaligned sequences, and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course was taught using Unix workstations, and participants were expected to be comfortable using the Unix operating system, programs, and a Unix text editor (programming knowledge was not required). The course was designed for biologists seeking advanced training in biological sequence analysis, for computer core directors and staff for molecular biology or genetics resources, and for scientists in other disciplines, such as computer scientists, who wished to survey current research problems in biological sequence analysis.

PARTICIPANTS
Aaronson, J., B.A., M.S., Merck Research Laboratories, New Jersey
Budarf, M., B.A., Ph.D., Children’s Hospital of Philadelphia
Butler, B., B.A., Ph.D., Genetics Computer Group, Inc., Wisconsin
Deng, Z., B.S., Ph.D., National Institutes of Health
Glusman, G., B.S., M.S., Weizmann Institute of Science, Israel
Guffanti, A., B.S., Telethon Institute of Genetics and Medicine, Italy
Hirakawa, M., B.S., M.S., Japan Information Center of Bio-science and Technology
Juvik, G., B.S., M.S., Stanford University, California
Kupfer, K., B.S., Ph.D., University of Texas Southwestern Medical Center
McCombie, W.R., B.A., Ph.D., Cold Spring Harbor Laboratory
Mishra, R., B.S., Harvard University Medical School
Mittur, A., B.S., University of Southern California
Peek, A., B.S., M.S., Rutgers University
Pelling, A., B.S., University of Portland, United Kingdom
Richardson, J., B.A., M.S., Ph.D., The Jackson Laboratory
Rosenberg, M., M.S., Ph.D., Massachusetts General Hospital
Santigo-Blay, J., University of Chicago, Illinois
Soriano, N., B.S., CNRS, France
Wang, G., M.S., M.D., University of Alabama
Winkler R., Ph.D., University of Arizona
Zhu, Z., B.S., Ph.D., University of Massachusetts Medical Center

SEMINARS
Eddy, S., Washington University School of Medicine, St. Louis, Missouri. Blocks, motifs, domains, and other protein databases. Multiple sequence comparison with hidden Markov models.
Gish, W., Washington University School of Medicine, St. Louis, Missouri. Statistics of sequence similarity scores. Searching databases with BLAST and friends. NCBI tools for sequence analysis.
Marr, T., Cold Spring Harbor Laboratory. Genome topographer.
Pearson, W., University of Virginia. Introduction and overview. Protein evolution-biology. Practical issues in protein sequence searching.
Smith, R., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania. Introduction to multiple sequence comparison.
Uberbacher, E., Oak Ridge National Laboratory. Analyzing eukaryotic genes with GRAIL.

Molecular and Cell Biology of S. pombe and Other Yeasts
November 6-19

INSTRUCTORS
Chappell, Thomas, Ph.D., University College London, United Kingdom
Rachubinski, Richard, Ph.D., McMaster University, Canada
Young, Paul, Ph.D., Queens University, Canada
Fission yeast is often chosen as a model system for studies of cell and molecular biology. This course was designed to introduce the basic genetic, molecular, and cell biological techniques necessary for investigators to adapt this system to their own laboratory/project. Topics included formal genetics and tetrad dissection, cytology including fluorescent and immunological techniques, transformation and gene replacement, plasmid recovery, and cell fractionation. Many of the experiments exploited mutant strains affected in cell cycle or cytoskeleton. Against this background, several other yeast systems of industrial or pathological interest were introduced. The Pichia system was used for protein expression, Yarrowia for cell fractionation studies, and Candida for hyphal transformation. In addition to the laboratory component, invited speakers addressed various aspects of the biology of these systems.

PARTICIPANTS
Bezbaruah, S., B.S., Ph.D., University College London, United Kingdom
Caviston, J., B.S., Fox Chase Cancer Center, Pennsylvania
Donald, A., B.S., Ph.D., Babraham Institute, United Kingdom
Drescher, R., B.S., M.S., University of Saarland, Germany
Fisher, R., B.A., M.D., Ph.D., University of California, San Francisco
Grishchuk, K., M.D., University of Colorado
Hurtado, C.A.R., B.S., University of Sao Paulo, Brazil
Kornacker, M., B.S., Ph.D., Bristol Myers Squibb, New Jersey
Kull, B., B.S., Ph.D., Max-Planck Institute, Germany
Nogi, Y., M.S., Ph.D., Saitama Medical School, Japan
Nose, H., B.S., Ph.D., Meiji Seika Kaisha, Ltd., Japan
Seno, G., B.S., M.S., Ph.D., Lilly Research Labs., Illinois
Severinov, K., M.S., Ph.D., Rockefeller University
Shida, H., B.S., Ph.D., National Research Council, Canada
Tohda, H., M.S., Asahi Glass Co., Ltd., Japan
Zhou, D., B.S., M.S., University of Alabama

SEMINARS
Chappell, T., University College London, United Kingdom.
Wandering along the secretory pathway of S. pombe.
Cregg, J., University of Oregon, Graduate Institute, Portland.
Development and general features of Pichia expression system. Pichia as a model system for studying peroxisome biogenesis.
Fink, G., Whitehead Institute, Cambridge, Massachusetts.
Dimorphism in yeast: A model for fungal development.
Leatherwood, J., State University of New York, Stony Brook.
Cell cycle regulation in S. pombe.

McLeod, M., State University of New York, Brooklyn. Mating type and meiosis in fission yeast. Role of ran kinases in meiosis.
Rachubinski, P., University of Alberta, Edmonton, Canada.
Mutants of peroxisome assembly in Yarrowia lipolytica.
Waterham, H., University of Oregon, Graduate Institute, Portland. Laboratory seminars.
Young, P., Queens University, Kingston, Ontario, Canada.
Sodium transport and salt tolerance in fission yeast.
Phage Display of Combinatorial Libraries

November 6-19

INSTRUCTORS
Barbas, Carlos, Ph.D., Scripps Research Institute
Burton, Dennis, Ph.D., Scripps Research Institute
Silverman, Gregg, Ph.D., University of California, San Diego

ASSISTANT
Briones, Amella, University of Strasbourg, France
Fuller, Roberta, Scripps Research Institute
Nayak, Jayakar, University of California School of Medicine, San Diego

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This library/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* were
also covered. The lecture series presented by a number of invited speakers focused on PCR of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in \textit{E. coli} and mammalian cells, antibody structure and function, catalytic antibodies, the whole biology of antibody activity, and recent results on the use of antibodies in therapy.

PARTICIPANTS

Bazinet, C., B.A., Ph.D., St. John's University.
Cahill, D., B.S., Ph.D., Max-Planck Institute, Germany
Gottstein, C., M.D., University of Texas Southwestern Medical Center
Hong, H.J., B.S., Ph.D., Korea Research Institute
Massey, R., B.A., University of Nevada
Momany, C., B.A., Ph.D., Purdue University, W. Lafayette, Indiana
Nathan, S., B.S., M.S., Ph.D., National University of Malaysia
Nilsson, L., M.S., Uppsala University, Sweden
Pasqualini, R., B.S., Ph.D., La Jolla Cancer Research Center, California
Phelps, J., B.A., Amylin Pharmaceuticals, Inc., California
Probert, W., B.S., Ph.D., Centers for Disease Control and Prevention, Colorado
Sompuram, S., B.S., M.S., Ph.D., Boston University School of Medicine
Steel, J., B.S., M.S., Imperial Cancer Research Fund, United Kingdom
Vazquez-Abad, D., B.S., M.D., University of Connecticut Health Center
Warren-Stewart, L., B.S., M.S., Pfizer, Inc., Connecticut
Yano, S., M.S., Ph.D., Yamanouchi Pharmaceutical Co., Ltd., Japan

SEMINARS

Kelsoe, G., University of Maryland School of Medicine. Biology of an antibody response.
Model, P., Rockefeller University. Biology of filamentous phage.
Nestler, P., Cold Spring Harbor Laboratory. Combinatorial chemistry.
Persson, M., Karolinska Hospital, Sweden. Eukaryotic expression of human antibodies isolated by phage display.
Scanlan, T., University of California, San Francisco. Catalytic antibody structure-function.
Scott, J., Simon Fraser University, Burnaby BC, Canada. Phage-displayed peptide libraries: Their construction, screening, and general use.
Siegel, D., University of Pennsylvania. Selection of anti-red cell antibodies by phage display.

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

- 5 Prime 3 Prime
- Amersham
- Andotek
- Applied Biosystems/P.E.
- Axon Instruments
- Beckman Instruments
- Becton-Dickenson
- Bio-101
- Bio-Rad
- Biometra
- Bioptechs, Inc.
- Boehringer Mannheim
- Brinkman Instruments
- Burleigh Instruments
- Carl Zeiss
- CBS Scientific
- Chroma Technology
- Codonics
- Corning Costar
- David Kopf Instruments
- Denton Vacuum
- Drummond Scientific
- DuPont NEN
- Epicentre Technologies
- Fisher Scientific
- FMC
- Folodyne
- Gatan
- General Valve
- Gibco/Life Technologies
- Hamamatsu
- Hoefer Scientific
- Instrument
- Invitrogen Corp.
- Isomedia Inc.
- Kodak
- Kramer Scientific Corp.
- Laser Science
- Leica
- LP Industries
- Mettler Greitz
- Millipore
- MJ Research
- Modulation Optics, Inc.
- Molecular Bioproducts
- Morrell Instruments, Inc.
- Narishige
- New England Biolabs, Inc.
- Nikon
- Novagen
- Novex
- Optronics Engineering
- Peltier
- Pharmacia
- Photometrics
- Princeton Separation
- Promega
- Qiagen
- Replicatch
- Savant Instruments
- Scion Image
- Seikagaku
- Siskiyou Design
- Spectra Physics
- Stratagene
- Sutter Instruments
- Trikinetics
- Tropix, Inc.
- Universal Imaging
- Vector Labs
- Vysis
- Wallac
Invited Speaker Program

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their latest findings on a weekly basis. These seminars keep the CSHL staff current on the latest developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

1996

January

Michael Lebl, Selectide Corporation. Combinatorial chemistry: From peptide to nonpeptide libraries. (Host: Peter Nestler)

Douglas Koshland, Carnegie Institution of Washington. Yeast chromosome structure is not an oxymoron. (Host: David Spector)

Sara Courtneidge, Sugen Inc. Src family kinases and the cell cycle. (Host: Nick Tonks)

Joseph Schlessinger, New York University School of Medicine. Cellular signaling by tyrosine phosphorylation. (Host: Terri Grodzicker)

February

Allen Oliff, Merck Research Laboratories. Farnesyl transferase inhibitors as anticancer agents. (Host: Michael Wigler)

Michael Stern, Yale University. FGF signaling pathways and cell migration guidance in C. elegans. (Host: Nick Tonks)

Gyorgy Buzsaki, Rutgers University, Center for Neuroscience. Network oscillations, irregular bursts, and plasticity in the hippocampal formation. (Host: Alcino Silva)

Derek van der Kooy, University of Toronto, Department of Anatomy and Cell Biology. What should learning and memory mutants look like? (Host: Michael Hengartner)

March

Robert Malenka, University of California, San Francisco. Mechanisms of long-term depression in the hippocampus. (Host: Roberto Malinow)

Rick Fidel, University of California, San Diego. Receptor-mediated pathways controlling Dictyostelium development. (Host: Hong Ma)

Ken Kemphues, Cornell University, Section of Genetics and Development. Establishing polarity in the C. elegans embryo: A PAR of the story. (Host: Michael Hengartner)

Arnie Levine, Princeton University, Department of Molecular Biology. The regulation of the p53 tumor suppressor gene. (Host: Terri Grodzicker)

April

Joseph Ecker, University of Pennsylvania, Department of Biology. The ethylene gas signaling pathway in plants. (Host: Hong Ma)

Steve Block, Princeton University, Department of Molecular Biology. Nanometers and piconewtons: Using optical tweezers to study biological motors. (Host: David Spector)

Yoshinori Watanabe, University of Tokyo, Department of Biophysics and Biochemistry. An RNA-binding protein Mei2 regulates the initiation and the process of meiosis in fission yeast. (Host: Akila Mayeda)


October

Randy Shekman, Department of Molecular and Cell Biology, University of California, Berkeley. Mechanism of cargo capture early in the secretory pathway. (Host: Kim Arndt)

Alan Hall, MRC Laboratory for Molecular Cell Biology, University College, London. Regulation of the actin cytoskeleton by RHO GTPases. (Host: Linda Van Aelst)

Ronald Plasterk, Netherlands Cancer Institute, Amsterdam. G protein function in C. elegans. (Host: Michael Hengartner)

November

Robert Tjian, University of California, Berkeley. Regulation of transcription in animal cells: Activators, co-activators, and the basal machinery. (Host: Terri Grodzicker)

December

Andrew McMahon, Harvard University. The role of cell signaling in patterning the vertebrate embryo. (Host: Grigori Enikolopov)

Timothy Bestor, Columbia University, Biology of genomic methylation patterns. (Host: Xiaodong Cheng)

Howard Schulman, Stanford University, Department of Neurobiology. Spatial and temporal regulation of CaM kinase II: An enzyme with a memory? (Host: Robert Malinow)
In-House Seminar Program

Cold Spring Harbor In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. They are particularly useful for research personnel who have recently joined the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

1996

January
Alcino Silva: Reverse genetics of learning and memory in mice.
Mirjana Maletic-Savatic (Malinow Lab): Dendritic exocytosis: A novel process linked to plasticity.

February
Michael Regulski (Tully Lab): NO: Genetic tools in Drosophila.
Rui-Ming Xu (Cheng Lab): The anatomy of casein kinase-1.
Hong Ma: To flower or not to flower: Functional analysis of AGAMOUS.
Alain Verreault (Stillman Lab): How cells make chromosomes.

March
Dong Jing Zou (Cline Lab): The role of CaMKII in visual system development.
Michele Cleary (Herr Lab): The Oct-1 POU domain: Taking on different shapes and different roles for regulating transcription.
Sanders Williams (Visiting Scientist): Myocyte nuclear factor: A novel transcriptional regulator involved in control of differentiation and proliferation of cells in the myogenic lineage.

April
Ulrich Grossniklaus: Sex or no Sex? The reproductivity biology of flowering plants.
Marie Luce Vignais (Gilman Lab): A JAK-STAT pathway in the PDGF response?
Yuri Lazebnik: Enemies within.
Maria Blasco (Greider Lab): Mouse as a model system for the study of telomerase.

October
Scott Lowe: Tumor suppressor genes and cellular defense mechanisms against cancer.
Michael Zhang (Marr Lab): Identification of protein-coding regions in the human genome based on quadratic discriminant analysis.

November
Winship Herr: Probing a biological machine in vivo: Genetic analysis of the basal transcriptional apparatus.
Linda Van Aelst: Regulators and effectors of Rac function.

December
Karl Giese (Silva Lab): Learning/memory of mutant mice: The next generation.

Andrew Flint (Tonks Lab): PTP1B, from structure to function.
Kathy O'Neill (Wigler Lab): RAS's CAP from yeast to human.
An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 462 students have participated in the course, and many have gone on to productive careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology; (2) an increased awareness of experimental approaches to science; (3) a deeper understanding of the major issues in the fields of biochemistry and genetics, and molecular and cellular biology; and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from over 280 applicants, took part in the program:

M. Nadeem Ali, Cambridge University
Advisor: Hollis Cline
Sponsor: Olney Foundation
NO and neuronal development.

Martha Betson, Cambridge University
Advisor: Linda Van Aelst
Sponsor: Frederica Von State Fund
Isolation of a full-length clone for POR3, a novel rac-binding protein.

Casey Blegen, University of Wisconsin
Advisor: Kim Arndt
Sponsor: National Science Foundation
Isolation of high-copy suppressors of the growth defect caused by overexpression of both SIT4 and SAP155.

Bilyana Georgieva, Mount Holyoke College
Advisor: Bruce Stillman
Sponsor: Burroughs Wellcome Fund
DNA replication: Construction and study of replication factor C (RFC) conditional mutants.
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Advisor</th>
<th>Sponsor</th>
<th>Project Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jennifer Gervais</td>
<td>Yale University</td>
<td>Gregory Hannon</td>
<td>Burroughs Wellcome Fund</td>
<td>The development of a gene-tagging retroviral technique using p53 and its transcription factors as a model system.</td>
</tr>
<tr>
<td>Jarret Glasscock</td>
<td>University of Arizona</td>
<td>Yi Zhong</td>
<td>National Science Foundation</td>
<td>CREB and the signal transduction pathway.</td>
</tr>
<tr>
<td>Michael Goller</td>
<td>Penn State University</td>
<td>Dr. Yuri Lazebnik</td>
<td>Burroughs Wellcome Foundation</td>
<td>Characterization of apoptosis-relevant endonuclease activity.</td>
</tr>
<tr>
<td>Christina Grozinger</td>
<td>McGill University</td>
<td>Winship Herr</td>
<td>Garfield Foundation</td>
<td>Determination of human cellular proteins interacting with the carboxyl terminus of HCF via the yeast two hybrid system.</td>
</tr>
<tr>
<td>Stephen Haggarty</td>
<td>University British Columbia</td>
<td>Bruce Futcher</td>
<td>Libby Internship</td>
<td>G₁ progression and the molecular basis of Start in the cell cycle of the yeast Saccharomyces cerevisiae.</td>
</tr>
<tr>
<td>Saul Kivimäe</td>
<td>Tartus University</td>
<td>Arne Stenlund</td>
<td>Glass Foundation</td>
<td>Interaction of papillomavirus E1 and E2 proteins at the viral origin of replication.</td>
</tr>
<tr>
<td>Tracy Litzl</td>
<td>Cedar Crest College</td>
<td>Erich Grotewold</td>
<td>National Science Foundation</td>
<td>PCR-based screening of a Mu grid in maize.</td>
</tr>
<tr>
<td>Valerie Maier</td>
<td>University of Glasgow</td>
<td>Richard McCombie</td>
<td>Curtis S. Read Foundation</td>
<td>Expression pattern analysis of open reading frames identified by the Schizosaccharomyces pombe genome sequence project.</td>
</tr>
<tr>
<td>Jonathan Montagu</td>
<td>Oxford University</td>
<td>Peter Nestler</td>
<td>Shakespeare Internship</td>
<td>The search for a potent and selective inhibitor of PTP-1B.</td>
</tr>
<tr>
<td>Teresa Niccoli</td>
<td>Cambridge University</td>
<td>Adrian Krainer</td>
<td>Jephson Educational Trust</td>
<td>Analysis of PRP 18-binding properties.</td>
</tr>
<tr>
<td>Betty Nyein</td>
<td>Massachusetts Institute of Technology</td>
<td>Jerry Yin</td>
<td>National Science Foundation</td>
<td>Characterization of the S162 mutation in CREB.</td>
</tr>
<tr>
<td>Viktoriya Paroder</td>
<td>SUNY, Stony Brook</td>
<td>David Beach</td>
<td>National Science Foundation</td>
<td>Enrichment and isolation of cDNAs from a known region of a chromosome.</td>
</tr>
<tr>
<td>Gerald Parvulus</td>
<td>Tuskegee University</td>
<td>Michael Hengartner</td>
<td>National Science Foundation</td>
<td>Temporal control of gene expression in the nervous system of the nematode, C. elegans.</td>
</tr>
<tr>
<td>Govindan Ramanathan</td>
<td>Rochester Institute of Technology</td>
<td>Grigori Enikolopov</td>
<td>Mr. and Mrs. Cornelius N. Bliss III</td>
<td>No synthase in the development of Drosophila melanogaster.</td>
</tr>
<tr>
<td>Gloria Jessica Salas</td>
<td>Florida International University</td>
<td>Ryuji Kobayashi</td>
<td>National Science Foundation</td>
<td>Isolation and sequencing of endoprotease Asp-N.</td>
</tr>
<tr>
<td>Joshua Silverman</td>
<td>University of California, San Diego</td>
<td>David Spector</td>
<td>Burroughs Wellcome</td>
<td>The study of structure-function relationships is one of the fundamentals of modern molecular biology.</td>
</tr>
<tr>
<td>Nathan Springer</td>
<td>Southeast Missouri State University</td>
<td>Rob Martienssen</td>
<td>Burroughs Wellcome</td>
<td>Molecular and developmental characterization of bladeless2, a Maize leaf development mutant.</td>
</tr>
<tr>
<td>Audrey Wells</td>
<td>University of New Mexico</td>
<td>Timothy Tully</td>
<td>National Science Foundation</td>
<td>Development of molecular-genetic tools in Drosophila.</td>
</tr>
</tbody>
</table>
The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, younger students can engage in introductory programs such as Nature Bugs, Nature Detectives, and Nature Discovery, and older students can enroll in more advanced programs such as Marine Biology and Nature Photography.

During the summer of 1996, a total of 397 students participated in 28 courses within the program. The classes were held outdoors, weather permitting, at the West Side School. The Laboratory has equipped and maintains classroom and laboratory facilities as well as a darkroom at West Side School. This facility is used as a base for the students' exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, Caumsett State Park, the Cold Spring Harbor Fish Hatchery and Aquarium, as well as other local preserves and sanctuaries.

In addition to the three, two-week sessions, the Adventure Education course meets on two Fridays for trips. The students go on a 10-mile bicycle hike to Sagamore Hill and a 12-mile canoe trip on the Nissequogue River.

PROGRAM DIRECTOR
William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR
Amy Anderson, Cold Spring Harbor Laboratory

INSTRUCTORS
Alison Forte, B.S. in Marine Science, University of Rhode Island
Donna Pandaliano, M.S., Science Teacher, Valley Stream School District
Linda Payoski, M.S., Science Teacher, Uniondale School District
Marjorie Pizza, M.S., Science Teacher, Locust Valley School District
Brian Withers, Fine Arts Teacher, New York City School System

COURSES
Nature Bugs
Nature Detectives
Nature Discovery
Ecology Explorers
Frogs, Flippers, and Fins
Pebble Pups
Freshwater Life
Seashore Life
Marine Biology
Nature Photography
Adventure Education
The year 1996 was a comparatively quiet one for Banbury Center, with 15 scientific meetings and 5 neurobiology courses, and the Conference Room being used on 12 other occasions. But the range of topics was broad and the quality of the meetings was high. Of the 492 scientists who came to the Center, 79 (16%) came from abroad, with scientists from the United Kingdom, Germany, and France predominating. Of the participants from the United States, most came from states with the highest concentrations of biomedical research institutions and companies, with four states (California, Maryland, Massachusetts, and New York) accounting for more than 50% of participants. Nevertheless, the geographical distribution was wide, with participants coming from 31 states. It is notable that no fewer than seven Nobel Laureates came to Banbury Center in 1996.

Another highlight of the year was, of course, the record snowfall of the 1995–1996 winter—73 inches here on Long Island—and we were fortunate to escape serious disruption to the 1996 program.

"Basic" Research

The 1996 meetings dealing with the molecular biology of the cell were outstanding in their coverage of fascinating processes. Two dealt with DNA, albeit in very different contexts. In 1995, Rich Roberts and Xiaodong Cheng described the interaction of a DNA methylase enzyme with a DNA molecule and showed that the cytosine base being methylated was "flipped" out of the double helix in a totally unexpected way. The meeting, DNA Base "Flipping": How and Why, reviewed new evidence that "base flipping" may be not be restricted to DNA methylases and may be a more general mechanism used by other enzymes for interactions with DNA molecules.

Another novel process in the cell is the means by which it replicates telomeres, the ends of the chromosomes. With the discovery of telomerase, the enzyme that makes telomeres, and data implicating telomeres in cancer and aging, this is a very "hot" area of research. Telomeres and Telomerase followed up on an historic Banbury Center meeting from 1994, and itself proved to have been held at just the right moment.

In contrast to telomeres and telomerase (a relatively new area of research), Mechanisms of Transcriptional Initiation discussed one of the oldest problems in molecular biology, namely, how RNA molecules are made from the appropriate genes and at the appropriate times. This was an extraordinary workshop, a veritable summit meeting of the world’s leading researchers. The schedule was also remarkable in being developed as the meeting progressed, with speakers limited to a few minutes and a few slides. It was very effective, a tribute to the hard work of the organizers, session chairs, and participants.

The remaining two meetings dealt with cells rather than molecules. Plant Reproductive Biology covered a range of topics of special interest to those breeding and developing new strains of plants. The meeting was a survey of recent research including processes such as cytoplasmic male sterility, self-incompatibility, and apomixis. Animal mesenchyme cells are fascinating, being involved in many developmental processes and having many different functions. The properties and roles of these cells were examined in Cellular and Molecular Biology of Mesenchyme. It was a great pleasure to work with Dan Marshak, a former scientist here at the Laboratory, in his new role of Director of Research for Osiris Therapeutics, Inc.

Human Genetics

Just a few years ago, it was found that the genetic abnormality in Huntington’s Disease was the presence of triplet repeats in the gene, leading to tracts of glutamines in the Huntingtin protein.
Such mutations had never been observed before, and this remarkable finding has become even more remarkable as other disorders with the same type of mutation have been discovered, all affecting the nervous system. The *Triplet Repeats and Polyglutamine Tracts* meeting brought together an eclectic group of scientists, not all of whom work on these disorders, to try to determine how to move the research from the genetic level to the biochemical and cellular levels. We were especially pleased to have Max Perutz attend and tell us of his work on a special type of protein-protein interaction.

**Manic-depressive Illness: Evolutionary and Ethical Issues** combined interesting scientific questions with societal issues. The scientific questions dealt with the adaptive advantages of mutations that are highly deleterious yet relatively common and widespread. The societal issues relating to the genetics of manic-depressive illness (MDI) are complicated by the apparent social benefits derived from some individuals afflicted by the illness. The meeting discussed what is known of the individual and social costs of MDI, and how these affect ethical questions regarding diagnosis.

**Vaccines**

In recent years, Banbury Center has been the site of several workshops on infectious diseases and vaccines funded by the Albert B. Sabin Vaccine Foundation. The Foundation supported two further meetings in 1996. *AIDS Vaccine Initiative*, held in March, reviewed the current state of affairs on developing an effective vaccine against HIV. This is a highly complex problem, in which economics and political considerations appear to play a role at least as large as scientific research. Fortunately, the organizers and the Foundation were able to involve individuals across a broad spectrum of interests who talked candidly about the difficulties involved.

The March meeting laid the groundwork for a second meeting in November, that was supported also by the National Institute of Allergy and Infectious Diseases, on *Case Studies in Vaccine Development*. Here participants analyzed in depth examples of vaccine development and production—both successful and unsuccessful—with a view to learning what works and what is to be avoided. It was a very interesting meeting, and instructive in dealing with real-life cases rather than abstract models.

**Neuroscience**

Neurobiology continues to play a large part in the Banbury Center year. There are the neurobiology courses in the summer, and in the autumn of 1996 there were also two neuroscience meetings.
One meeting brought together studies of two rather different systems. On the one hand, glutamate receptors are known to play key roles in nerve and synapse development, while on the other, there is the phenomenon of synaptic plasticity in which synaptic activity produces long-lasting changes. *Plasticity of Glutamate Receptors* examined whether new data on the functioning of glutamate receptors can help in understanding the mechanisms underlying synaptic plasticity.

Synaptic plasticity may be important in learning and in the establishment of memory. *Genetic Approaches to Learning and Memory* reviewed recent advances in finding genes involved in learning and memory. In addition, participants covered some of the social issues involved in the establishment of a new field of research; in this case, the use of transgenic mice to study the genetic basis of learning and memory. These issues include developing fruitful relationships between two groups of researchers—molecular biologists and neurophysiologists—that have not interacted before, and establishing methodological standards.

**Biotechnology**

This year, only one Banbury Center meeting was directly concerned with a biotechnology topic. Some years ago, there was great optimism that DNA molecules could be used as therapeutic agents. But while the DNA molecule is versatile, its properties are not what the molecular biologist needs to modify gene expression in living cells. *Modified Nucleic Acids: Chemistry and Applications* examined the ways in which DNA molecules can be chemically modified so that they retain their properties but are easier to get into cells and are more stable when they are there. Participants included organic chemists—specialists at making new molecules—and molecular biologists who are trying to use these compounds.

**The Executives’ Conference**

This series of meetings has become legendary for the variety of topics covered, and for the extraordinarily high quality of both scientist-speakers and executive-guests. This year’s meeting was no exception and perhaps had an added benefit in that the topic, *Human Development*, was unfamiliar to many at the meeting. Even more so than usual, we asked the participants to go on a long intellectual journey with us: from patterning in the embryo, through human embryonic development and the development of gender differences, to the fertilization and manipulation of human embryos in vitro. I was delighted to have two long-time friends participate—Lewis Wolpert, who published the seminal paper on pattern formation in embryos, and Robert Winston, who developed preimplantation genetic testing, and who now, as Lord Winston, speaks in the House of Lords on these issues.

"*Education*" for Nonscientists

Banbury Center has for many years held workshops on modern biomedical research for groups of nonscientists, but this year, we did something rather special when some 20 federal and state judges came to Banbury for a 10-day workshop on *The Art of Judging: Perspectives of Science*. Funded by the Federal Judicial Center in Washington, D.C., this was a most interesting workshop, covering not just genetics, but also statistics in the courtroom, risk assessment, and the history and philosophy of science. A highlight of the meeting was a talk by Laurie Garrett based on her international best seller, *The Coming Plague*. The workshop received very high ratings from the participants and we expect that the Federal Judicial Center will return in 1997.

One of the most important and influential series of meetings ever held at Banbury Center was that funded by the Alfred P. Sloan Foundation. Over a period of 12 years, the Sloan Foundation pro-
vided funds to invite Congressional staff and science journalists to Banbury for workshops on biomedical research that has an immediate impact on the public. These workshops were very highly regarded by the participants and provided us with invaluable connections in organizations as varied as the Congressional Research Service and the National Association of Science Writers. Cold Spring Harbor Laboratory Public Affairs and Banbury Center decided that these workshops were so valuable that we should do at least one more, and so the *Science Journalists Workshop on Genetics of Human Behavior* took place in November. The entire meeting dealt with controversial issues and we were fortunate in having the leading researchers come to us, including Dean Hamer, who was the first to find an association between a genetic locus and male homosexuality.

**Courses at Banbury Center**

As usual, Banbury Center, during the summer months, was host to five courses, organized by the Laboratory's Meetings Office: *Genetic-Epidemiological Studies of Complex Diseases; Neurobiology of Human Neurological Disease; Computational Neuroscience: Vision; Neurobiology: Brain Development and Function;* and *Advanced Drosophila Genetics.*

**Other Meetings**

Banbury Center is such a wonderful facility that we are glad to make it available to the local community. In 1996, the Center was used for one-day retreats by the Lloyd Harbor Conservation Board, Cold Spring Harbor School District, Family Service League, and Huntington Hospital. In addition, Susan Cooper of Public Affairs organized a Lloyd Harbor seminar for residents of the Village.

**Banbury Center on the World Wide Web**

The Internet, and particularly the World Wide Web (WWW), has become an indispensable tool for all scientists, including myself. Through it, I can locate people; find out what research they do and where it has been published; and send letters and invitations via e-mail to participants in our meet-

Robertson house (left) and Sammis Hall (right) provide housing accommodations at Banbury Center.
ings. It was inevitable, then, that Banbury Center would have its own web site. Here, we publish our annual report; provide descriptions of the Center and the style of our meetings; and important information such as maps and directions on how to reach us. A link to our web pages can be found on the Laboratory's home page at www.cshl.org.

Funding

It is impossible to overstate the importance of the contributions made by members of the Cold Spring Harbor Laboratory Corporate Sponsor Program to the Banbury Center. Funds from this Program continue to be essential for a large part of the Banbury Center's activities and provide a firm basis for the rest of our program. In 1996, there were seven meetings for Corporate Sponsors, an increase due to the success of the Plant Associate Program that provided funds for a plant science meeting on Plant Reproductive Biology. Other Corporate Sponsor meetings were Plasticity of Glutamate Receptors; DNA Base "Flipping": How and Why; Telomeres and Telomerase; Triplet Repeats and Polyglutamine Tracts; and Modified Nucleic Acids: Chemistry and Applications.

Companies in the biotechnology world continue to be important sponsors of meetings on topics of special interest to them. All such proposals received must satisfy the same high standards of scientific interest and relevance to the Laboratory's range of research and educational interests—we do not merely rent out the Conference Center—and the meetings are organized in the same way as all our meetings. In 1996, Osiris Therapeutics funded the meeting on Cellular and Molecular Biology of Mesenchyme, while Geron Corporation contributed to the meeting on Telomeres and Telomerase.

J.P. Morgan, Inc. was again very generous in its support of the Executive Conference on Human Development, enabling us to invite scientists of the highest caliber and providing a wonderful ambience in which to listen to and ponder the very best of modern biomedical research.

Foundations and other nonprofit organizations made significant contributions to our year. Our long association with three Foundations led to four meetings. The Albert B. Sabin Foundation supported the two meetings relating to vaccines: AIDS Vaccine Initiative and Case Studies in Vaccine Development; the Charles A. Dana Foundation provided funds for Manic-depressive Illness: Evolutionary and Ethical Issues; and the Hereditary Disease Foundation contributed to the meeting Triplet Repeats and Polyglutamine Tracts. The meeting on Genetic Approaches to Learning and Memory was funded by the Marie H. Robertson Memorial Fund for Neurobiology. Finally, the Federal Judicial Center supported the fascinating meeting for federal and state judges.

Acknowledgments

The staff of the Center—Bea Toliver and Ellie Sidorenko in the Conference Center office and Katya Davey in Robertson House—did a wonderful job in making sure that all aspects of the meetings went smoothly. Chris McEvoy and Andy Sauer ensured that the Banbury Center estate continues to be a beautiful place. Art Brings and the Facilities Department, and Jim Hope and his Food Service staff were unfailingly helpful, especially as more demands are made on their staff as the Laboratory's meetings program continues to grow. And, last but not least, my thanks to the scientists at the Laboratory for their continuing support of the Center.

Jan Witkowski

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MEETINGS

Cellular and Molecular Biology of Mesenchyme

February 11–February 14

FUNDED BY

Osiris Therapeutics, Inc.

ARRANGED BY

D.R. Marshak, Osiris Therapeutics, Inc., Baltimore, Maryland

SESSION 1: Skeletal
Chairperson: D.K. Heinegard, University of Lund, Sweden

A.I. Caplan, Case Western Reserve University, Cleveland, Ohio: The mesengenic process and its relationship of skeletogenesis and hematopoiesis.


M.W. Long, University of Michigan, Ann Arbor: Bone-marrow-derived human osteoprogenitor cells.

G.A. Rodan, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania: Osteoblast requirement for osteoblast formation in culture.

D.K. Heinegard, University of Lund, Sweden: Macromolecule markers of chondrocyte and osteoblast development.

R. St-Arnaud, Shriners Hospital, Montreal, Quebec, Canada: Abnormal bone development in mice with targeted mutations in the gene for 25-hydroxyvitamin-D-24-hydroxylase.

B. DeCrombrugghe, M.D. Anderson Cancer Center, Houston, Texas: Approaches to chondrocyte and osteoblast differential.

SESSION 2: Marrow
Chairperson: B. Torok-Storb, The Fred Hutchinson Cancer Research Center, Seattle, Washington

C.I. Civin, The Johns Hopkins Oncology Center, Baltimore, Maryland: Lessons from lymphohematopoiesis.

P.J. Quesenberry, University of Massachusetts Medical Center, Worcester: Marrow stromal and cytokine regulation of primitive marrow stem cells.

S.L. Gerson, Case Western Reserve University, Cleveland, Ohio: Mesenchymal stem cells as a gene therapy target.

M.A. Thiede, Osiris Therapeutics, Inc., Baltimore, Maryland: Reinfusion of mesenchymal stem cells for stromal reconstitution following lethal irradiation.


SESSION 3: Muscle
Chairperson: C. Ordahl, University of California, San Francisco

C. Ordahl, University of California, San Francisco: Early skeletal muscle development.

E.N. Olson, University of Texas Southwestern Medical Center, Dallas: bHLH factors as regulators of somatogenesis.

B. Wold, California Institute of Technology, Pasadena: Multiple pathways to skeletal muscle.

L.A. Leinwand, University of Colorado at Boulder: Specialized function or redundancy in muscle gene families.


H.M. Blau, Stanford University, California: Muscle-mediated gene therapy.

L.A. Leinwand, E.N. Olson, C. Ordahl
SESSION 4: Vasculature
Chairperson: W.H. Burgess, American Red Cross/Holland Laboratory, Rockville, Maryland

B. Christ, Anatomisches Institut, Freiburg, Germany: Development of the embryonic vascular system.
W.H. Burgess, American Red Cross/Holland Laboratory, Rockville Maryland: FGF-1 and mitogenic signal transduction: Targeting specificity.
D. Bowen-Pope, University of Washington, Seattle: Role of PDGF in connective tissue development and function.

SESSION 5: Connective Tissue/Neural Crest/Other
Chairperson: D.R. Marshak, Osiris Therapeutics, Inc., Baltimore, Maryland

W.E. Wright, University of Texas Southwestern Medical Center, Dallas: Telomeres, aging, and the senescence of stem cells.
D.M. Noden, College of Veterinary Medicine, Cornell University, Ithaca, New York: Lineage analyses of craniofacial mesenchymal populations.
J.M. Lauder, University of North Carolina, Chapel Hill: Regulation of embryonic craniofacial mesenchyme differentiation by serotonin.

SESSION 1: Modified Backbones
Chairperson: D. Cook, ISIS Pharmaceuticals, Inc., Carlsbad, California

M.H. Caruthers, University of Colorado, Boulder: Chemical synthesis and biochemical studies with dithioate and boranephosphate DNA.
A. Eschenmoser, Laboratorium fur Organische Chemie, Zurich, Switzerland: Pyranosyl-RNA.
P.P. Herdwijn, Rega Institute, Leuven, Belgium: Backbone modifications resulting in strong hybridizing oligonucleotides.
S.A. Benner, ETH-Zentrum, Zurich, Switzerland: Modified nucleobases; modified sugars; modified linkers.
SESSION 2: Novel Nucleobases  
Chairperson: S. Benner, ETH-Zentrum, Zurich, Switzerland

F. Seela, Universitat Osnabruck, Germany: The control of DNA structure by modified purines.
D.E. Bergstrom, Purdue University, West Lafayette, Indiana: "Wild-card" bases: Design and synthesis.
E.T. Kool, University of Rochester, New York: Altering the functional properties of DNA without modifying the backbone.
T.S. Widlanski, Indiana University, Bloomington: Expanding the genetic backbone; synthesis of sulfonate-modified nucleic acids and their interactions with polymerases.

M. Matteucci, Gilead Sciences, Inc., Foster City, California: Covalent and noncovalent conformational restriction of oligonucleotides.
M. Waring, University of Cambridge, United Kingdom: Moving the purine 2-amino group around: Effects on sequence recognition and ligand binding.

SESSION 3: Functional Oligos  
Chairperson: A. Eschenmoser, ETH-Zentrum, Zurich, Switzerland

R.L. Letsinger, Northwestern University, Evanston, Illinois: Controlling properties of oligonucleotides by chemical modifications.
J. Sun, INSERM, CNRS, Paris, France: Oligonucleotide-directed triple helix formation: Stabilization and extension of recognition sequences of triple helices.

M. Frank-Kamenetskii, Boston University, Massachusetts: Sequence-specific targeting of duplex DNA with peptide nucleic acid (PNA).
D.H. Turner, University of Rochester, New York: Binding of oligonucleotides to the catalytic site of a group I ribozyme.
O.D. Scharer, Harvard University, Cambridge, Massachusetts: The use of modified nucleic acids to study DNA repair enzymes.

SESSION 4: Physical Chemistry and Structure  
Chairperson: R. Letsinger, Northwestern University, Evanston, Illinois

K.J. Breslauer, Rutgers University, Piscataway, New Jersey: Nucleic acid hybridization, stability, and ligand-binding properties: A thermodynamic perspective.

J. Feigon, University of California, Los Angeles: Solution structures of DNA triplexes containing modified nucleotides.
S.M. Freier, ISIS Pharmaceuticals, Inc., Carlsbad, California: Modified antisense oligonucleotides: Hybridization pharmacokinetics and pharmacology.

SESSION 5: Gene Therapeutic Leads  
Chairperson: J. Feigon, University of California, Los Angeles

S. Gryaznov, Lynx Therapeutics, Inc., Hayward, California: Synthesis and physicochemical properties of oligonucleotide phosphorodiamidates.
P.E. Nielsen, The Panum Institute, Copenhagen, Denmark: PNA (peptide nucleic acids). What have we learned and where does it lead?

P.D. Cook, ISIS Pharmaceuticals, Inc., Carlsbad, California: Making drugs out of oligonucleotides.
H.E. Moser, Ciba-Geigy Ltd., Basel, Switzerland: Modified antisense oligonucleotides: From structure to biological activity in animals.
AIDS Vaccine Initiative: The Sabin Foundation's Role

March 24–March 26

FUNDED BY

Albert B. Sabin Vaccine Foundation

ARRANGED BY

M.R. Hilleman, Merck Research Institute, West Point, Pennsylvania
M.L. Clements, The Johns Hopkins University, Baltimore, Maryland

SESSION 1: Challenges
Chairperson: M.R. Hilleman, Merck Research Institute, West Point, Pennsylvania

M. R. Hilleman, Merck Research Institute, West Point, Pennsylvania: AIDS Vaccine Initiative: Challenges and directions in targeted research.
R. Kurth, Paul-Ehrlich-Institut, Langen, Germany: Perspective of the European community.

SESSION 2: Identifying Obstacles and Opportunities in Research on HIV Vaccines
Facilitator: L.A. Miller, Intermedica, Inc., Norwalk, Connecticut

SESSION 3: Federal and Foundation Initiatives
Chairperson: D. Bolognesi, Duke University Medical Center, Durham, North Carolina

J. Killen, NIAID, National Institutes of Health, Bethesda, Maryland: Perspective of NIAID.
D. Bolognesi, Duke University Medical Center, Durham, North Carolina: The problems that confront the evolution of the Vaccine Initiative as seen from the OAR deliberations.

Panel and General Discussion: Future Roles of Government and Foundations
Moderator: L.A. Miller, Intermedica, Inc., Norwalk, Connecticut
D. Henderson, Johns Hopkins University, Baltimore, Maryland
K.I. Shine, National Academy of Sciences, Washington, D.C.
J. Lederberg, The Rockefeller University, New York, New York

SESSION 4: How Can the Sabin Foundation Best Organize Its Efforts to Increase the Probability of Developing an Effective HIV Vaccine?
Chairperson: M.L. Clements, Johns Hopkins University School of Public Health, Baltimore, Maryland

H.R. Shepherd, Albert B. Sabin Vaccine Foundation, New Canaan, Connecticut: The resources of the Sabin Foundation over the next three years.

Building a Consensus: Parts 1 and 2
Facilitator: L.A. Miller, Intermedica, Inc., Norwalk, Connecticut
M.L. Clements, Johns Hopkins University School of Public Health, Baltimore, Maryland: Conclusions: Drawing up a list of goals.
DNA Base "Flipping": How and Why

April 7–April 10

FUNDED BY
Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY
X. Cheng, Cold Spring Harbor Laboratory
R.S. Lloyd, University of Texas Medical Branch, Galveston, Texas

SESSION 1: Biophysical Considerations of Base Flipping
Chairperson: D.J. Patel, Memorial Sloan-Kettering Cancer Center, New York, New York
D.J. Patel, Memorial Sloan-Kettering Cancer Center, New York, New York: Bulge and loop conformations in RNA.
D.G. Gorenstein, University of Texas Medical Branch, Galveston: NMR structure of a dG base flipping in a benzo(a)pyrene diol epoxide duplex adduct.
M. Goodman, University of Southern California, Los Angeles: Evidence for the occurrence of ionized base mispairs during DNA synthesis.
L.S. Beese, Duke University Medical Center, Durham, North Carolina: Structure of DNA polymerase-DNA complexes.

SESSION 2: DNA Methyltransferases
Chairperson: X. Cheng, Cold Spring Harbor Laboratory
G. Schluckebier, Freie Universitat Berlin, Germany: Three-dimensional structure of adenine specific DNA-methyltransferases from Thermus aquaticus.
G.I. Verdine, Harvard University, Cambridge, Massachusetts: Keepers of the code: Studies on proteins that decorate and mend the genome.
X. Cheng, Cold Spring Harbor Laboratory: Hhal methyltransferase.
S. Klimasauskas, Institute of Biotechnology, Vilnius, Lithuania: Stopped-flow fluorescence studies of methyltransferase-induced base-flipping in DNA.
E. Weinhold, Max-Planck-Institut fuer Molekulare Physiologie, Dortmund, Germany: Evidence for a base flipping mechanism by the adenine-specific DNA methyl-transferase from Thermus aquaticus.

H.H Thorp, R.J. Roberts
SESSION 3: DNA Repair Enzymes
Chairperson: R.S. Lloyd, University of Texas Medical Branch, Galveston

S. Wallace, University of Vermont, Burlington: Processing of oxidative DNA base lesions.

J.A. Tainer, The Scripps Research Institute, La Jolla, California: Crystal structures and mutational analysis of DNA base-excision repair enzymes: Structural basis for specificity and catalysis.

R.P. Cunningham, State University of New York, Albany: Novel motifs for substrate recognition by the repair enzyme endonuclease III.

K. Morikawa, Protein Engineering Research Institute, Osaka, Japan: Crystal structure of pyrimidine dimer excision-repair enzyme complexed with DNA: A novel flipping-out mechanism.

H.-W. Park, Duke University Medical Center, Durham, North Carolina: The structure of DNA photolyase: Insights into substrate binding mode.

R.S. Lloyd, University of Texas Medical Branch, Galveston: Mechanism of endonuclease V.

L. Grossman, The Johns Hopkins University, Baltimore, Maryland: DNA structures induced by RNAP as 'start' sets for DNA repairs.

SESSION 4: DNA Repair Enzymes

R. Savva, University College London, United Kingdom: Further investigations of the mode of action of uracil-DNA glycosylase.

D.W. Mosbaugh, Oregon State University, Corvallis: Structure, function, and interaction of E. coli uracil-DNA glycosylase with the Ugi protein.

B. Demple, Harvard University School of Public Health, Boston, Massachusetts: Is there a role for base flipping in AP endonucleases?

A.-L. Lu-Chang, University of Maryland, Baltimore: Interaction of mismatch-containing DNA with E. coli MutY and mammalian MutY homologs.

T. Ellenberger, Harvard Medical School, Boston, Massachusetts: Crystal structure of E. coli.

SESSION 5: DNA Trans-actions
Chairperson: S.M. Linn, University of California, Berkeley

S.M. Linn, University of California, Berkeley: Other DNA repair processes that might relate to base flipping.

H.H. Thorp, The University of North Carolina at Chapel Hill: Detecting single base mismatches: Consequences of base flipping.

P.C.E. Moody, University of Leicester, United Kingdom: Why should O6-MeG-DNA methyltransferases flip?


B.L. Bass, HHMI, University of Utah, Salt Lake City: Double-stranded RNA adenosine deaminase.

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Plant Reproductive Biology

April 21–April 24

FUNDED BY Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY R. Pruitt, Harvard University, Cambridge, Massachusetts

SESSION 1: Male Reproductive Development I
Chairperson: P.S. Schnable, Iowa State University, Ames


S. McCormick, Plant Gene Expression Center, Albany, California: Cell division mutations in Arabidopsis pollen development.

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D. Twell, University of Leicester, United Kingdom: Gene regulation and cell determination in male gametophyte development.

W.Z. Cande, University of California, Berkeley: Chromosome behavior during meiotic prophase in maize meiocytes.

S.H. Strauss, Oregon State University, Corvallis: Floral homeotic genes as tools for engineering complete reproductive sterility in Populus and other forest trees.
SESSION 2: Male Reproductive Development II
Chairperson: M. Albertsen, Pioneer Hi-Bred International, Johnston, Iowa

C.S. Levings III, North Carolina State University, Raleigh: The Texas male-sterile cytoplasm of maize.
P.S. Schnable, Iowa State University, Ames: Fertility restoration of cytoplasmic male sterility: Molecular analysis of the r2 restorer of cmsT maize.
S. Mackenzie, Purdue University, West Lafayette, Indiana: Tissue-specific expression of a mitochondrial sequence influences pollen development in cms bean.
M. Hanson, Cornell University, Ithaca, New York: Cytoplasmic male sterility in Petunia.

SESSION 3: Female Reproductive Development
Chairperson: H. Dickinson, University of Oxford, United Kingdom

J.L. Bowman, University of California, Davis: Molecular genetics of carpel development.
J.A. Verbeke, University of Arizona, Tucson: Fusion events during gynoecium development.
C.S. Gasser, University of California, Davis: Genetic analysis of ovule development.
U. Grossniklaus, Cold Spring Harbor Laboratory: Enhancer detection as a tool to study reproductive development in Arabidopsis.

SESSION 4: Evolution of Reproductive Systems
Chairperson: U. Grossniklaus, Cold Spring Harbor Laboratory

C.F. Crane, Texas A&M University, College Station: Developmental implications of apomixis.
R. Bicknell, Crop & Food Research Ltd., Christchurch, New Zealand: Development of Hieracium as a model system to study apomixis.
A.M. Chaudhury, CSIRO Division of Plant Industry, Australia: Fertilization-independent seed development.

SESSION 5: Self-incompatibility/Fertilization
Chairperson: A.M. Chaudhury, CSIRO, Canberra City, Australia

H. Dickson, University of Oxford, United Kingdom: Defensin-like proteins in the pollen coating of Brassica.
T.-H. Kao, Pennsylvania State University, University Park: Role of Petunia receptor kinase PRK1 in pollen and embryo sac development.

J.M. Herr, Jr., University of South Carolina, Columbia: A new perspective of ovule and female gametophyte evolution.
A. Ray, University of Rochester, New York: Floral initiation and ovule development.
R. Fischer, University of California, Berkeley: Control of ovule development in Arabidopsis.

W.E. Friedman, University of Colorado, Boulder: Comparative and phylogenetic approaches to reconstructing the evolution of plant reproductive patterns.
S.D. Russell, University of Oklahoma, Norman: Structural considerations in angiosperm fertilization.
C. Dumas, Ecole Normale Superieure, Lyon, France: How to investigate fertilization in flowering plants.
Triplet Repeats and Polyglutamine Tracts

May 5–May 8

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program and the Hereditary Disease Foundation

ARRANGED BY

D. Housman, Massachusetts Institute of Technology, Cambridge
D. Nelson, Baylor College of Medicine, Houston, Texas
A. Tobin, University of California, Los Angeles
N.S. Wexler, College of Physicians & Surgeons of Columbia University, New York, New York

SESSION 1: Clinical Presentation

N.S. Wexler, College of Physicians & Surgeons of Columbia University, New York, New York

SESSION 2: Triplet Repeat Diseases

A.B. Young, Massachusetts General Hospital, Boston: Interaction of polyglutamines with mitochondrial import proteins or leader sequences.
J.F. Gusella, Massachusetts General Hospital, Charlestown: Huntington’s disease.
C. Ross, The Johns Hopkins University, Baltimore, Maryland: HD, DRPLA.
J.M. Vance, Duke University Medical Center, Durham, North Carolina: Similarities of the GAG trinucleotides and the uniqueness of the nervous system in neurogenetics.

SESSION 3: Polyglutamine Tracts

M. Perutz, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Incorporation of glutamine repeats in a small protein causes irreversible association into oligomers.
K. Stott, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: The oligomerization mechanism of a model protein with engineered glutamine repeats.

D.L. Nelson, Baylor College of Medicine, Houston, Texas: Fragile X syndrome update.
P. Patel, Baylor College of Medicine, Houston, Texas: The Friedreich’s ataxia intronic GAA expansion: What does it do?
K. Taneja, University of Massachusetts Medical Center, Worcester: Detection of triplet repeats by in situ hybridization.

Discussion: Triplet repeat disorders.
SESSION 4: Protein-Protein Interactions

S. Fields, University of Washington, Seattle: Yeast hybrid methods to analyze protein-protein and RNA-protein interactions.

T. Dawson, Johns Hopkins University School of Medicine, Baltimore, Maryland: Expansion of polyglutamine repeat in Huntington leads to abnormal proteins interactions involving calmodulin.

J.R. Burke, Duke University Medical Center, Durham, North Carolina: Possible roles for GAPDH in polyglutamine repeat diseases.

B. Koshy, Baylor College of Medicine, Houston, Texas: Characterization of ataxia-1 and its interactions with GAPDH.

R. Brent, Massachusetts General Hospital, Boston: Assigning functions to proteins by mapping connections.

Discussion: Where next in studying protein-protein interactions?

SESSION 6: Cell Death

N. Heintz, HHMI, Rockefeller University, New York, New York: Cell death and the cell cycle.

S. Lowe, Cold Spring Harbor Laboratory: Modulation of apoptosis in development and cancer therapy.

M. Hengartner, Cold Spring Harbor Laboratory: Programmed cell death in C. elegans nervous system.

H. Dudek, Children’s Hospital, Boston, Massachusetts: Signal transduction pathways and the regulation of neuronal survival and death.

Discussion: What do cell death studies offer research on triplet repeat diseases?

SESSION 5: Mouse Models

J.-L. Mandel, IGBMC, Illkirch, France: Progress in construction of cellular or mouse models of Huntington’s disease.

G. Bates, Guy’s Hospital, London, United Kingdom: Transgenic models. Phenotype observed in mice transgenic for the Huntington’s disease mutation.

M.E. MacDonald, Massachusetts General Hospital, Charlestown: Making models of the unstable Huntington’s disease CAG repeat in the mouse.

D.E. Merry, University of Pennsylvania School of Medicine, Philadelphia: Androgen receptor transgenic mice: Attempts at creating a mouse model for spinal and bulbar muscular atrophy.

Discussion: Future developments using transgenic models.

SESSION 7: Looking for Therapies


A. Tobin, University of California, Los Angeles: Round Table Discussion: How to move from ‘basic’ research to ‘therapeutic’ research?

N.S. Wexler, College of Physicians & Surgeons of Columbia University, New York, New York

L.J. DeGennaro, Wyeth-Ayerst Research, Princeton, New Jersey

C.T. Caskey, Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania

G.D. Yancopoulos, Regeneron Pharmaceuticals, Inc., Tarrytown, New York

Plasticity of Glutamate Receptors: Cellular and Molecular Mechanisms

October 6–October 9

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

R. Malinow, Cold Spring Harbor Laboratory

R.L. Huganir, HHMI, Johns Hopkins University School of Medicine, Baltimore, Maryland

SESSION 1: Formation of a Model Synapse: The Neuromuscular Junction

Chairperson: R.L. Huganir, HHMI, Johns Hopkins University School of Medicine, Baltimore, Maryland

S.C. Froehner, University of North Carolina School of Medicine, Chapel Hill: Syntrophins: Modular adaptor proteins in synaptic signaling.

J.R. Fallon, Brown University, Providence, Rhode Island: Regulation of synaptic structure.
SESSION 2: Structure and Function of Glutamate Receptors
Chairperson: R. Malinow, Cold Spring Harbor Laboratory

R.W. Gereau, The Salk Institute for Biological Studies, San Diego, California: Mechanism of desensitization of mGluR5.
P.H. Seeburg, University of Heidelberg, Germany: Genetic regulation of glutamate receptor properties.
M. Mishina, University of Tokyo, Japan: Synaptic plasticity in mutant mice lacking glutamate receptor channels.

S. Nakanishi, Kyoto University Faculty of Medicine, Japan: Distinct signaling calcium of different metabotropic receptor subtypes.
M. Hollmann, Max Planck Institute for Experimental Medicine, Gottingen, Germany: Functional domains and topology of glutamate receptors.

SESSION 3: Physiological Properties of Glutamate Receptors
Chairperson: R.A. Nicoll, University of California, San Francisco

S.G. Cull-Candy, University College London, United Kingdom: Single-channel diversity and dependence on subunit composition in the cerebellum.
M.L. Mayer, LCMN, NICHD, National Institutes of Health, Bethesda, Maryland: Permeation and block in GluR channels.
R.W. Tsien, Stanford University School of Medicine, California: Understanding the quantal response at glutamater-gic synapses between hippocampal neurons.

G.L. Westbrook, Vollum Institute, Oregon Health Sciences University, Portland: Intracellular regulation of NMDA receptors.
C.E. Jahr, Vollum Institute, Oregon Health Sciences University, Portland: Regulation of glutamate channel activation at synapses.

SESSION 4: Regulation of Glutamate Receptor Function
Chairperson: R.C. Malenka, University of California, San Francisco

R.L. Huganir, HHMI, Johns Hopkins University School of Medicine, Baltimore, Maryland: Regulation of glutamate receptors by protein phosphorylation.
M.W. Salter, Hospital for Sick Children, Toronto, Canada: NMDA receptor for regulation by tyrosine phosphorylation.

T.R. Soderling, Vollum Institute, Oregon Health Sciences University, Portland: Regulation of glutamate receptors by Cam-Kinase II.

SESSION 5: Synaptic Structure and Localization of Glutamate Receptors
Chairperson: P.H. Seeburg, University of Heidelberg, Germany

P. Somogyi, Oxford University, United Kingdom: Location of glutamate receptors in relation to transmitter release sites.
R.J. Wenthold, NIDCD, National Institutes of Health, Bethesda, Maryland: Distribution and targeting at glutamate receptors in neurons.

K.M. Harris, Children's Hospital, Boston, Massachusetts: Structural diversity of hippocampal glutamatergic synapses.
M.H. Sheng, HHMI, Massachusetts General Hospital, Boston: Molecular organization of glutamatergic synapses.
SESSION 6: Plasticity at Excitatory Synapses I
Chairperson: S.G. Cull-Candy, University College London, United Kingdom

G.L. Collingridge, University of Bristol School of Medical Sciences, United Kingdom: Glutamate receptors and LTP in the hippocampus.

D.M. Kullmann, Institute of Neurology, London, United Kingdom: Long-term potentiation of AMPA and NMDA receptor-mediated signals in the hippocampus.


H. Cline, Cold Spring Harbor Laboratory: Parallel mechanisms underlying "development" and "plasticity" of synaptic connections.

R.C. Malenka, University of California, San Francisco: Bidirectional control of synaptic strength.

D.W. Choi, Washington University School of Medicine, St. Louis, Missouri: mGlur modulation of excitotoxic neuronal death.

SESSION 7: Plasticity of Excitatory Synapses II
Chairperson: S.C. Froehner, University of North Carolina School of Medicine, Chapel Hill

R.A. Nicoll, University of California, San Francisco: The role of metabotropic glutamate receptors in hippocampal mossy fiber transmission.

T. Takahashi, Brain Research Institute, Tokyo, Japan: Mechanisms underlying short-term and long-term synaptic modulation induced by presynaptic glutamate receptors.

D.J. Linden, Johns Hopkins University School of Medicine, Baltimore, Maryland: A protein-synthesis-dependent late phase of cerebella long-term depression.

SESSION 8: Global Mechanisms
Chairperson: M.L. Mayer, LCMN, NICHD, National Institutes of Health, Bethesda, Maryland

M.F. Bear, HHMI, Brown University, Providence, Rhode Island: Bidirectional synaptic plasticity and its regulation in the cerebral cortex.

J.E. Lisman, Brandeis University, Waltham, Massachusetts: A new role for NMDA channels in memory recall.

The Art of Judging: Perspectives of Science

October 10–October 15

Funded by
The Judiciary Leadership Development Council, The Federal Judicial Center, and Cold Spring Harbor Laboratory

Arranged by
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
J.D. Watson, Cold Spring Harbor Laboratory: Frontiers of genetic research.

SESSION 2

SESSION 3
P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Special policy issues involving DNA and genetic engineering.
D. Hull, Northwestern University, Chicago, Illinois: The process of science: The system of rewards.
SESSION 4
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: DNA and the Human Genome Project.
D. Wilkinson, Princeton University, New Jersey: Evidence, errors, and proof in science: Cosmology as a paradigm.

SESSION 5
D. Micklos and Mark Bloom, DNA Learning Center, Cold Spring Harbor Laboratory: Visit to Cold Spring Harbor Laboratory and personal DNA experiments.

SESSION 6
L. Moses, Stanford University Medical School, California: Statistics and probability in science.
S.A. Schaffer, New York University, New York: Research integrity and scientific misconduct.

SESSION 7
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Eugenics.

SESSION 8
M. Gallo, Robert Wood Johnson Medical School, Piscataway, New Jersey: Toxicology and the environment.

SESSION 9
J.W. Hicks, Alabama Department of Forensic Sciences, Birmingham: Science and criminal investigations.
P.M. Eisenberger, Princeton University, New Jersey: Role of universities in science and technology in 21st century.

Manic-depressive Illness: Evolutionary and Ethical Issues

October 20–October 23

FUNDED BY
Charles A. Dana Foundation

ARRANGED BY
K.R. Jamison, The Johns Hopkins School of Medicine, Baltimore, Maryland
R. Cook-Deegan, National Academy of Science, Washington, D.C.
L.L. Hall, National Alliance for the Mentally Ill, Arlington, Virginia
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Overview of Manic-depressive Illness; Status of Genetic Studies; Individual and Social Costs of Manic-depressive Illness
Co-Chairpersons: P.C. Whybrow, University of Pennsylvania, Philadelphia, and L.L. Hall, National Alliance for the Mentally Ill, Arlington, Virginia

K.R. Jamison, The Johns Hopkins School of Medicine, Baltimore, Maryland: Overview of manic-depressive illness: Its cost and benefits.
R.J. DePaolo, The Johns Hopkins School of Medicine, Baltimore, Maryland: Status of genetic studies.


SESSION 2: Adaptive Value of Manic-depressive Illness
Chairperson: S.H. Barondes, University of California, San Francisco

R. Richards, University of California, San Francisco: Creativity in manic-depressives and their first-degree relatives.
D.R. Wilson, University of Cincinnati, Ohio: Evolutionary advantages of mood instability.

P. Gilbert, Kingsway Hospital, Derby, United Kingdom: Evolutionary perspectives on depression.

SESSION 3: Evolutionary Biology: Perspectives from Other Conditions
Chairperson: R. Cook-Deegan, National Academy of Science, Washington, D.C.

R.M. Neese, University of Michigan Medical School, Ann Arbor: Disease in general, including sickle cell, thalassemia-malaria, and CF-cholera.
D. Hamer, National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Homosexuality: The findings.

L.M. Silver, Princeton University, New Jersey: Genetics of complex behavioral traits (in mouse).

SESSION 4: Evolutionary Perspectives on Manic-depressive Illness: Implications
Chairperson: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Focused discussion: What's at stake?

Focused discussion: Does manic-depression have adaptive value?
Leader: S.H. Barondes, University of California, San Francisco

Focused discussion: Is there a tradeoff between social benefit and individual harm?

SESSION 5: Development of Consensus Statement
Co-Chairpersons: R. Cook-Deegan, National Academy of Sciences, Washington, D.C., and K.R. Jamison, The Johns Hopkins School of Medicine, Baltimore, Maryland

C. Burr, R.J. DePaolo
D. Hamer
J.P. Morgan & Co. Incorporated/Cold Spring Harbor Laboratory
Executive Conference on Human Development

October 25-October 27

ARRANGED BY

J.D. Watson, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

L. Wolpert, University College and Middlesex Hospital Medical School, London, United Kingdom: How embryos develop.

SESSION 2

B. Hogan, Vanderbilt University Medical School, Nashville, Tennessee: Human development from conception to birth
D. Page, Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Genes and sex.
J. Hall, University of British Columbia, Vancouver, Canada: When development goes awry.

SESSION 3

R. Rosenfeld, Oregon Health Sciences University, Portland: Human growth and maturation: Significance, biology, and therapeutic interventions.

SESSION 4

A. Silva, Cold Spring Harbor Laboratory: Studying learning and memory.
T. Marr, Cold Spring Harbor Laboratory: Genetics and manic-depressive illness.

SESSION 5

M. Hines, University of California, Los Angeles: Developmental differences between the sexes.
R. Winston, Royal Postgraduate Medical School, London, United Kingdom: Controlling human development: Ethical issues.
Telomeres and Telomerase

November 3–November 6

FUNDED BY
Cold Spring Harbor Laboratory Corporate Sponsor Program with additional support from Geron Corporation

ARRANGED BY
T. de Lange, The Rockefeller University, New York, New York
C. Greider, Cold Spring Harbor Laboratory

SESSION 1: Telomere Length Regulation I
Chairperson: C. Greider, Cold Spring Harbor Laboratory
E.H. Blackburn, University of California, San Francisco: Yeast and other telomerases.
V. Lundblad, Baylor College of Medicine: EST1, EST2, EST3, and EST4/CDC13: In vivo regulators of yeast telomerase.

SESSION 2: Telomere Length Regulation II
Chairperson: T.R. Cech, HHMI, University of Colorado, Boulder
R. Wel linger, University of Sherbrooke, Canada: Studies on the terminal DNA structure of eukaryotic telomeres.
K.W. Runge, Cleveland Clinic Foundation Research Institute, Ohio: Telomere length regulation in Saccharomyces cerevisiae.
V.A. Zakian, Princeton University, New Jersey: Telomere maintenance in Saccharomyces.
A.J. Lustig, Tulane Medical Center, New Orleans, Louisiana: A novel mechanism for telomere size control in yeast.
D.M. Shore, University of Geneva, Switzerland: Telomere length regulation by Rap1 protein in yeast.

SESSION 3: Telomerase
Chairperson: E.H. Blackburn, University of California, San Francisco
K. Collins, University of California, Berkeley: Architecture of the telomerase RNP.
D.E. Shippen, Texas A&M University, College Station: DNA recognition and synthesis by ciliate and plant telomerases.
D. Romero, University of Minnesota, Minneapolis: The fidelity of Paramecium telomerase in vivo.
F. Muller, University of Fribourg, Switzerland: Telomeres in nematodes.
F. Ishikawa, Tokyo Institute of Technology, Yokohama, Japan: Cloning of a mammalian telomerase component gene.

SESSION 4: Drosophila Telomeres
Chairperson: C.S. Newlon, UMDNJ-New Jersey Medical School, Newark
H. Biessmann, University of California, Irvine: Drosophila and mosquito telomerases.

T.R. Cech, M.-L. Pardue
SESSION 5: Telomeric Chromatin
Chairperson: T.D. Petes, University of North Carolina, Chapel Hill

D. Rhodes, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: The crystal structure of the DNA-binding domain of yeast Rap1 in complex with telomeric DNA.
S. Schultz, University of Colorado, Boulder: Structure of the O. nova telomere binding protein complexed with ssDNA.
J.P. Langmore, Biophysics Research Division, University of Michigan, Ann Arbor: Functional studies of vertebrate model telomeres in vitro, and determination of the covalent terminal structure of telomeres from mortal and immortal human cells.

SESSION 6: Telomeres and Telomerase in Cancer and Aging
Chairperson: V.A. Zakian, Princeton University, New Jersey

C. Greider, Cold Spring Harbor Laboratory: Telomerase in mouse models.
R.R. Reddel, Children’s Medical Research Institute, Wentworthville, Australia: Lengthening of telomeres in human cell lines without detectable telomerase activity.
J.W. Shay, University of Texas Southwestern Medical Center, Dallas: Telomerase and cancer: Diagnostic, prognostic, and therapeutic implications.

Science Journalists Workshop on Genetics of Human Behavior

November 7–November 9

Funded by
Cold Spring Harbor Laboratory

Arranged by
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Human Behavior

M.G. McInnis, Johns Hopkins Hospital, Baltimore, Maryland: Psychiatric disorders as aberrant behavior.
D. Goldman, NIAAA, National Institutes of Health, Rockville, Maryland: Biological and genetic approaches to understanding alcoholism.
M. Linnoila, NIAAA, National Institutes of Health, Bethesda, Maryland: Biological and genetic approaches to understanding aggressive behavior.
D. Hamer, National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Genetic analyses of homosexuality.
T. Tully, Cold Spring Harbor Laboratory: Genetic studies of learning and memory.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: The new human genetics.

SESSION 2: DNA Experiment

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: DNA fingerprinting using PCR.
Case Studies in Vaccine Development

November 17–November 20

FUNDED BY
Albert B. Sabin Vaccine Foundation and the
National Institute of Allergy
and Infectious Diseases

ARRANGED BY
R.G. Douglas, Merck & Co., Inc., Whitehouse Station, New Jersey
B. Gellin, NIAID, National Institutes of Health, Bethesda, Maryland

SESSION 1: Orientation and Objectives
B.G. Gellin, National Institutes of Health, Bethesda, Maryland: Case studies in vaccine development: Concept and goal of the workshop.
R.G. Douglas, Merck & Co., Inc., Whitehouse Station, New Jersey: The vaccine R&D "system."
L. Galambos and J.E. Sewell, The Johns Hopkins University, Baltimore, Maryland: Perspectives on networks: Lessons learned from other industries.

SESSION 2: Case Studies in Vaccine Development I
Ty21a
Introduction: M.M. Levine, University of Maryland, Baltimore

SESSION 3: Case Studies in Vaccine Development II
Rotavirus vaccine
Introduction: S. Plotkin, Pasteur Merieux Connaught, Marnes la Coquette, France (via teleconference)
Varicella vaccine
Introduction: A. Gershon, College of Physicians & Surgeons of Columbia University, New York, New York

SESSION 4: Case Studies in Vaccine Development III
RSV vaccine
Introduction: C. Hellman, National Institutes of Health, Bethesda, Maryland
Hib vaccine
Introduction: J.I. Ward, UCLA Center for Vaccine Research, Torrance, California

SESSION 5: Case Studies in Vaccine Development IV
Hepatitis vaccines
Introduction: M.R. Hilleman, Merck Research Laboratories, West Point, Pennsylvania
Cold-adapted influenza vaccine
Introduction: P.F. Wright, Vanderbilt Medical Center, Nashville, Tennessee

SESSION 6
The vaccine R&D fabric: Common and uncommon threads
Discussion Leader: R.G. Douglas, Merck & Co., Inc., Whitehouse Station, New Jersey
The vaccine R&D fabric: A view from the outside
Discussion Leaders: L. Galambos and J.E. Sewell, The Johns Hopkins University, Baltimore, Maryland

SESSION 7
Lessons learned and lessons taught: Strengthening the vaccine R&D system
Discussion Leaders: B.G. Gellin and R. Rabinovich, National Institutes of Health, Bethesda, Maryland, and P.K. Russell, The Johns Hopkins University, Baltimore, Maryland
Genetic Approaches to Learning and Memory

December 8–December 11

Funded by
Marie H. Robertson Memorial Fund for Neurobiology

Arranged by
E.R. Kandel, College of Physicians & Surgeons of Columbia University, New York, New York
A. Silva, Cold Spring Harbor Laboratory
S. Tonegawa, Massachusetts Institute of Technology, Cambridge

Session 1: Calcium Calmodulin Kinase and Plasticity
K.P. Giese, Cold Spring Harbor Laboratory: The role of the autophosphorylation at T286 of α-CaMKII.
K. Fox, University of Wales, Cardiff, United Kingdom: The role of α-CaMKII in barrel cortex plasticity.
M.P. Stryker, University of California, San Francisco: Plasticity mechanisms responsible for cortical development: Comparison of findings in vivo and in vitro in mouse visual cortex.
J.O. McNamara, Duke University Medical Center, Durham, North Carolina: Kindling: An NMDA receptor-dependent plasticity of mammalian nervous system.

Session 2: Genetics, Synapses, and Learning
Chairperson: S. Nakanishi, Kyoto University Faculty of Medicine, Japan
T.C. Sudhof, University of Texas Southwestern Medical Center at Dallas: Mechanisms of neurotransmitter release.
H. Thoenen, Max Planck Institute for Psychiatry, Martinsried, Germany: Gene targeting and virus-medicate gene transfer in the analysis of neuronal plasticity.
H.-S. Shin, Pohang University of Science and Technology, Pohang, Republic of Korea: IP3 Kinase mutation, LTP, and learning.
P.F. Chapman, University of Wales, Cardiff, United Kingdom: Behavioral and physiological analyses of a transgenic model of Alzheimer’s disease.
H.-P. Lipp, University of Zurich, Switzerland: Biological relevance of behavioral effects observed in knockout mice: Natural selection studies.
S. Tonegawa, Massachusetts Institute of Technology, Cambridge: NMDA receptor-dependent synaptic plasticity is needed for spatial memory study with CAI-restricted knockout mice.
M.A. Wilson, Massachusetts Institute of Technology, Cambridge: Dissecting the role of hippocampal plasticity in spatial representations using region-specific genetic knockouts.

Round Table Discussion: Genetics and cognition: What will we need to make the connection?
Introduced by: M.P. Stryker, University of California, San Francisco

Session 3: LTP and Beyond
Chairperson: S. Tonegawa, Massachusetts Institute of Technology, Cambridge
A. Silva, Cold Spring Harbor Laboratory: Plasticity, spikes, and learning.
R. Morris, UMDS Guy’s Hospital, London, United Kingdom: Selective inhibition of LTP in the dentate gyrus in vivo does not affect spatial learning in mice lacking Thy-1.
E.G. Abel, College of Physicians & Surgeons of Columbia University, New York: Genes important for long-term memory.
D.R. Storm, University of Washington, Seattle: Role of the adenylyl cyclases and cAMP for learning and memory.
T.J. O’Dell, University of California, Los Angeles: The role of protein kinase A and modulatory neurotransmitters in low frequency stimulation-induced LTP.
SESSION 4: Neural Mechanisms and Behavior
Chairperson: S.F. Heinemann, The Salk Institute, San Diego, California

S. Nakanishi, Kyoto University, Japan: Glutamate receptor function in neuronal plasticity.
S.G.N. Grant, University of Edinburgh, United Kingdom: Postsynaptic tyrosine kinase signaling.
R.F. Lathe, University of Edinburgh, United Kingdom: Genes selectively expressed in hippocampus.
S. Itohara, Kyoto University, Japan: Astrocytes and neuronal plasticity.
M. Picciotto, Yale University School of Medicine, New Haven, Connecticut: Pharmacological and behavioral effects of a mutation in the high-affinity receptor for nicotine.

Round Table Discussion: Genetic background and the study of behavior.
Introduced by: T. Tully, Cold Spring Harbor Laboratory

SESSION 5: Ion Channels and Learning
Chairperson: H. Thoenen, Max Planck Institute for Psychiatry, Martinsreid, Germany

P.A. Slesinger, University of California, San Francisco: Genetic manipulations of G protein-gated inwardly rectifying potassium channels.
S.F. Heinemann, The Salk Institute, San Diego, California: Mutations in glutamate receptor genes.
J. Roder, Samuel Lunenfeld Research Institute, Toronto, Canada: Behavioral LTP analysis of various GluR knockouts.

Round Table Discussion: Genetic background of ES cells.
Introduced by: The Jackson Laboratory, Bar Harbor, Maine
Mechanisms of Transcriptional Initiation

December 14–December 17

FUNDED BY
Cold Spring Harbor Laboratory

ARRANGED BY
W. Herr, Cold Spring Harbor Laboratory
R. Kingston, Massachusetts General Hospital, Boston
K. Yamamoto, University of California, San Francisco

TOPIC: What are the functional targets of activators?

SESSION 1: Activator Targets: GTFs and Holoenzyme I
Chairpersons: R. Losick, Harvard University, Cambridge, Massachusetts and
T. Maniatis, Harvard University, Cambridge, Massachusetts

SESSION 2: Activator Targets: TAFs
Chairpersons: C. Gross, University of California, San Francisco and
B. Stillman, Cold Spring Harbor Laboratory

TOPIC: What is the mechanism of activator function? I

SESSION 3: Chromatin: Transcriptional Activation and Modification
Chairpersons: E. O'Shea, University of California, San Francisco and
R. Treisman, Imperial Cancer Research Fund, London, United Kingdom

SESSION 4: Stabilization and Isomerization of the Preinitiation Complex
Chairpersons: S. Adhya, National Cancer Institute, Bethesda, Maryland and
A. Hochschuld, Harvard Medical School, Boston, Massachusetts

TOPIC: What is the mechanism of activator function? II

SESSION 5: Promoter Clearance, Elongation, and Phosphorylation
Chairperson: K. Yamamoto, University of California, San Francisco

SUMMARY AND OVERVIEW
T. Maniatis, Harvard University, Cambridge, Massachusetts
Introduced by: E. Simpson, The Jackson Laboratory, Bar Harbor, Maine

M. Ptashne

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In 1989, we first brought local 5th and 6th grade students into our Bio2000 Teaching Laboratory for a summer genetics camp, entitled Fun With DNA. That educational experiment tested the notion of whether or not young students are capable of understanding the Gene Age that they will inherit. The answer can be found in our 1996 visitation statistics—for the first time, middle school students (grades 5–8) replaced high school students as the DNALC’s largest clientele for lab instruction.

A 1990 grant from the National Science Foundation supported our first organized effort to train middle-school faculty. A 1991 grant from the Hearst Foundation allowed us to recruit elementary educators Jane Conigliaro and Diane Jedlicka and to begin to rigorously implement programs for Long Island schools. Our first goal was to establish a substantial summer camp program, including Fun With DNA and its follow-up, World of Enzymes. Portledge School was added as a summer camp site to provide easier access for students from Nassau County. With later support from the Howard Hughes Medical Institute and the Barker Welfare Foundation, summer camps were conducted for minority students from Manhattan at the American Museum of Natural History and A. Phillip Randolph High School.

Genetics as a Model for Whole Learning was initiated in 1992, as the umbrella for our middle school activities. The program provides DNALC staff support to help school systems implement a selection of laboratories from the Fun With DNA and World of Enzymes camps. The object is to train 5th and 6th grade classroom teachers to use genetics as the hub of an across-discipline network to link science with math, social studies, current events, and language arts. Genetics as a Model for Whole Learning incorporates numerous educational features that are compatible with national and local science standards:

- age-appropriate instruction according to cognitive development
- principles from current research on learning and memory
- progressive sequencing of activities (constructivism)
- discovery approach accommodates a variety of learning styles (visual, auditory, tactile)
- interdisciplinary approach accommodates multiple intelligence types
- cooperative learning and problem solving
- active learning and creative behaviors
metaphorical and associative learning
science as a way of looking at the world and solving problems
skills and strategies for life-long learning
the museum as a school
research technology and equipment
applying content expertise to analysis of current issues and ethical
decision-making
introducing new career opportunities

Thus far, we have implemented the program in 12 member districts of the Curriculum Study Program. The logistics of setting up and running independent Genetics as a Model for Whole Learning programs at so many distant sites is extremely complex. DNA Learning Center staff meet with the district administrators and classroom teachers to design a customized program—ranging from intensive instruction for several classes to a briefer exposure for all students in a grade level. DNA Learning Center staff then provide 10–15 days of faculty training and in-school instruction. Over a 2–3 year period, teachers become comfortable with the curriculum and implement their own customized units, typically culminating with a laboratory field trip to the DNALC. Equipment and supplies for several different laboratories are transported between schools. Logistics became even more complex this year, when we added ten elementary/middle schools in Community School District 29, near Kennedy Airport, and Corlears Junior High School in Chinatown. All told, the 1996 program involved 5,045 students and 138 teachers in 38 schools.

Above: Andrew Morotti instructs students in the Genetics as a Model for Whole Learning Program. Right: Students examine fruit fly mutations with a compound microscope.
An Historic Opportunity to Resynthesize Early Biology Education

The response to *Genetics as a Model for Whole Learning* has been almost overwhelming. Word of the program is now spreading in the New York metropolitan area, and we have a waiting list of districts interested in joining the program when space becomes available. The program is also attracting the interest of educators in other parts of the country. With donations from Tom White, Research Vice President of Roche Molecular Systems, Jane Conigliaro has conducted *Fun With DNA* and *World of Enzymes* camps for the Point Arena School District in Mendocino County, California. High school faculty trained through our National Science Foundation Leadership Institute (1993–1995) have provided in-service training in genetics for approximately 1000 elementary/middle school faculty and initiated summer camp programs in several states.

The demand for high-quality science instruction for younger students was further accelerated by the long-awaited publication this year of the National Science Education Standards. This document challenges school districts nationwide to provide all precollege students with hands-on, minds-on experiences that encourage critical thinking in science. The Science Standards, in combination with two educational trends, should specifically focus attention on the 6th grade as the major objective in the battle to build a genetically literate populace. First, school restructuring is moving 6th grade classes out of the elementary school and into the middle school. With this move, 6th grade teachers, accustomed to teaching all subjects with the same class all day, now are forced to specialize by content area. Some must make the conversion to science teacher, even though the typical K-6 certification received by most teachers has virtually no science requirements. Second, the New York City School Board recently decreed that life science should be moved to the 6th grade, followed by physical science in the 7th grade and earth science in the 8th.

We are thus seeing in the nation’s largest metropolitan area a whole generation of 6th grade teachers who need retraining for their new roles as life science teachers. This is an historic opportunity to start afresh with an entirely new curriculum that views life processes through the lens of genetics. This would immediately bring early biology education into line with modern research. Students rising into high school with higher expectations in biology would, in turn, force more sweeping changes in entrenched high school curricula. *Genetics as a Model for Whole Learning* takes a large step in this direction and at the same time builds upon former classroom teachers’ strengths in reading and across-discipline integration.

Renewed Support from the Hearst Foundation

With this opportunity ahead of us, we were pleased to receive news at year’s end of a $100,000 grant from the Hearst Foundation to support the further expansion of *Genetics as a Model for Whole Learning*. Hearst support will be used to take the curriculum from its current photocopied notebook form to a published text. In addition to hands-on laboratories and supporting discussion, the formalized curriculum will include a fully elaborated “case study” of human growth hormone, based on our exhibit, *Story of a Gene*. A teacher’s guide will include student learning objectives, content connections, lab prep, and answers to questions. The lab text will be augmented by multimedia learning materials, including the
Genetic Computer Arcade and Internet-accessible support. The DNA Learning Center's track record for producing articulate, well-timed, and user-friendly texts—plus established contacts in the publishing industry—should help us to identify a suitable publishing partner.

With formalization of the curriculum, we will be prepared to initiate a national training program based on dissemination methods developed with National Science Foundation support over the last decade. Teacher training will utilize our nationwide network of university and precollege collaborators with whom we have organized teacher training in the past and who are committed to modeling new approaches to genetics instruction. We will especially target institutions that have received precollege education grants from the Howard Hughes Medical Institute, which encourages collaborations between grantees. Piggybacking onto established precollege programs makes cost-effective use of existing infrastructures for reaching teachers, interacting with school decision-makers, and providing ongoing teacher follow-up and support. Hearst support will also be used to foster linkages between high schools with existing laboratory programs and elementary/middle schools that are implementing Genetics as a Model for Whole Learning. In this way, elementary through high school faculty can collaborate to establish sequenced instruction that incorporates hands-on learning about genetics at several stages in child and adolescent development.

Strategic Alliances to Reach to Minority and Disadvantaged Students

We have always been honest about our efforts to contribute solutions to the problems of providing excellent science education for minority and disadvantaged students. Our location among wealthy villages of Long Island's "Gold Coast" puts the largest numbers of needy students at least a half-hour commute from our center. To defeat this distance problem, we have attempted to develop strategic alliances that allow us to have a presence in less affluent areas of metropolitan New York. In 1996, we initiated key collaborations to better reach minority and disadvantaged students in Brooklyn and Queens—the two boroughs of New York City that are physically located on Long Island.

Especially satisfying has been the development of a close collaboration with Dr. Mort Slater, who directs Gateway to Higher Education, a program of the City University of New York Medical School, to stimulate minority achievement in science. Dr. Slater operates student science enrichment programs serving 1500 students at five New York City High Schools: Brooklyn Technical, Erasmus Hall, Jamaica, Fort Richmond, Queens Gateway, and John F. Kennedy. Dr. Slater's uncanny ability to find ways around the arcane bureaucracy of New York City Public Schools is the perfect complement to our content expertise in modern biology. Thus, working together with Principal Lee McCaskill, Assistant Principal Andrea Canner, and Science Teacher Judy Cohen, we have been able to establish a modern DNA Laboratory at Brooklyn Tech. One of New York City's three specialized science high schools with competitive admission, Brooklyn Tech has recently begun to broaden its scope beyond its traditional engineering emphasis. The DNA Laboratory will immediately upgrade biology instruction, allowing implementation of the recommended molecular genetics laboratories in seven Advanced Placement Biology classes in spring 1997 and a molecular genetics elective in the 1997–1998 academic year.

Equally important has been a growing collaboration with Community School
District 29, a public school system in Queens serving 25,000 students in grades K-8. Located immediately to the north of Kennedy Airport, District 29 serves a population that is 95% minority, where two thirds of students are eligible for lunch assistance and 31% have limited English proficiency. Working closely with Superintendent Celestine Miller, Director of Funded Programs Ellen Schlesinger, and Supervisor of Science Diane Erhlich, we began the large-scale implementation of Genetics as a Model for Whole Learning. The initial year’s program involved 24 days of in-school instruction and 30 field trips to the DNALC that involved 1000 students. Again joining forces with Dr. Slater, we intend to establish a genetics laboratory at the district’s new Gifted and Talented Academy for Arts and Sciences at Intermediate School 59. The new laboratories at Brooklyn Tech and IS59 will provide a large-scale test of integrated genetics instruction developed and tested in the mini-districts of Long Island’s north shore. The laboratories will serve as satellite learning centers from which we can extend student enrichment and faculty development to schools throughout Brooklyn and Queens.

Students from Community School District 29 during a visit to the Learning Center.

We have devoted considerable resources to Central Islip School District, located in the middle of Long Island. With a population composed of equal parts Black, Hispanic, and White students, Central Islip represents the changing face of urban America. Here, we have assisted science teacher Jerry Watkins to develop an advanced biology program that mirrors those offered by larger or wealthier districts. Jerry offers a molecular genetics elective during the academic year and a summer research program. In 1996, he also taught two sessions of DNA Science at the American Museum of Natural History. At the middle school level, Lynn Casdia continues to teach summer camps based on Fun With DNA and World of Enzymes.

Our Bio2000 Laboratory Has Reached Capacity

We have always considered the Bio2000 Laboratory the heart of our operation. It was the first space renovated and opened for student instruction in spring 1988, six months in advance of our official opening. It has remained in continuous operation since that time, even during major building renovations that closed the remainder of the public facilities in 1993-1994. We began operation with a morning lab session for high school students, which became fully booked within a year. An afternoon session was added for middle school students in 1991. This year, a 30% increase was achieved by adding a second afternoon session on Monday
and Friday. Now, with double or triple booking virtually every school day between October 1 and June 15, we can honestly say that the Bio2000 Laboratory has reached saturation. Although we can likely increase in-school instruction considerably over the next year, this will be extremely hard on staff and will also increase demand for field trips to the DNALC. Thus, further significant expansion of laboratory experiences must await the hoped-for BioMedia Addition to the south of our building.

The DNALC teaching facilities were also fully occupied throughout the summer months. Laboratory workshops were offered at four levels: Fun With DNA (5th to 8th grade), World of Enzymes (6th to 8th grade), DNA Science (9th to 12th grade), and Advanced DNA Science (10th to 12th grade). Sue Lauter initiated a new course, Introduction to Computer Design, held in the BioMedia Laboratory. Demand for summer instruction remained high, with 338 students participating at courses held at the DNALC, as well as in the Beckman teaching labs on the main CSHL campus and at Portledge School in Locust Valley. Support from the Howard Hughes Medical Institute and the Barker Welfare Foundation allowed us to extend opportunities for 99 students to attend courses conducted at venues in New York City: the American Museum of Natural History and A. Philip Randolph High School. Significantly, minority students composed 40% of summer workshop participants.

It is also worth noting that during 1996, we saw our 100,000th visitor. Although this would be a yearly or even quarterly statistic for a large science center, the numbers alone do not tell the whole story. We are extremely proud of the fact that more than half of our clientele has received an intensive laboratory experience that goes well beyond the standard museum visit. A student lab exposure at the DNALC averages two hours, whereas off-site instruction averages six hours per student. Workshops average 30 hours of instruction.

With the opening of our own World Wide Web site (http://darwin.cshl.org), we can now count "virtual" visitors among our clientele. By year's end, the site was receiving about 10,000 "hits" per month, including visitors from more than 50 countries. We quickly deployed substantial animations of molecular genetic processes, using newly-released Shockwave compression software. The current animations, showing DNA analysis by polymerase chain reaction and Southern blotting, are the most popular stops at our site. These animations are a unique resource for understanding dynamic molecular events and cannot be found elsewhere. We intend to greatly expand our animation gallery in 1997.

### DNA Learning Center Visitation 1988–1996

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Cablevision's multimedia production, *Long Island Discovery*, continued as a popular attraction, drawing more than 10,000 viewers. The 12th annual *Great Moments in DNA Science* Honors Student Seminar Series drew 575 local students and teachers. Speakers and topics were:

- Scott Lowe, CSHL: Cancer and Cell Suicide.

**Teacher Training Workshops Bear Brunt of Shift in NSF Funding**

With support from the National Science Foundation (NSF) and the Howard Hughes Medical Institute, we continued our effort to make human DNA fingerprinting by polymerase chain reaction (PCR) practical for widespread use in biology classes. The *Human Genome Diversity-Student Allele Database* is a mini-lab unit based on an experiment from our *Laboratory DNA Science* book that allows students to examine their own DNA polymorphisms. During the academic year, Mark Bloom and collaborator John Kruper (now at Allegheny College) introduced the unit to 98 college faculty through a series of four NSF-sponsored workshops.
The precursor of the published text, *DNA Science*, used in 1987. The Vector Van, pictured "on-the-road," transported all equipment and supplies necessary to instruct the *DNA Science* curriculum.

The program is based on an *Alu* insertion polymorphism, an example of a "jumping gene" that has amplified itself to over 500,000 copies in the human genome. A small subset of *Alu* insertions have occurred recently in our evolutionary past and can be used as biological clocks to study human origins. Students can use their own allelic data to conduct a case study in population genetics. The data can be manipulated using *Student Allele Database* software developed by the DNALC and the University of Chicago. Accessible via the Internet, the *Student Allele Database* allows students to tabulate *Alu* insertion data, test Hardy-Weinberg equilibrium, and compare two populations by contingency Chi-square and genetic distance. The database includes *Alu* allele frequencies from relic populations around the world and from nearly 900 students who have performed the experiment at the DNALC. Additional data sets are being added by the classes of faculty trained through the NSF program.

The DNALC continued its international leadership in 1996, conducting teacher workshops in Australia and Italy. In January, Dave Micklos traveled to Melbourne to conduct a *DNA Science* Workshop that drew 50 secondary teachers from throughout Victoria, Australia. The course was jointly organized by Suzanne Cory, director of the Walter and Eliza Hall Institute, and James Pittard, Chairman of the Microbiology Department at the University of Melbourne. A May course on "DNA Amplification and Genetic Diversity," sponsored by the Porto Conte Foundation, introduced the *Student Allele Database* project to 20 secondary teachers from the Naples area. The workshop was organized by our collaborator Marcello Siniscalco and Giuseppe Martini, of the International Institute of Genetics and Biophysics.

It was with some nostalgia that Dave Micklos conducted *DNA Science* Institutes at Eastern Mennonite University (Harrisonburg, VA) and the University of Alaska (Fairbanks), concluding a 10-year NSF training program for high school biology teachers. *DNA Science* is the nation's longest-running training program in molecular genetics for high school faculty and has been in continuous operation since its founding in 1985. DNALC staff have instructed more than 1500 fac-
ulty at institutes in 38 states and Puerto Rico, and an estimated 2000 additional faculty nationwide have been trained by other academic groups using the *DNA Science* curriculum. Thus, there is good reason to believe that the *DNA Science* curriculum has been the major training mechanism to introduce precollege biology teachers to DNA manipulation techniques. But this achievement seemed for naught when we received word at year’s end that, after two successive proposal attempts, NSF would not renew support for the *DNA Science* program. We were very lucky to have maintained NSF support over an entire decade, considering that 3-5-year funding is the norm. The DNALC’s high school training was always an anomaly in the NSF portfolio—we were the only program funded explicitly to take faculty training to distant sites in other states. In that sense, we always led a fragile existence.

The DNALC’s training programs developed in an era when NSF supported training programs were operated by individual investigators at major universities and research institutions. However, several years ago, NSF initiated a major program of “systemic initiatives”—multi-million dollar awards to state and urban education authorities. While the theory of improving science education within a whole school system is attractive, many see these as essentially redundant entitlement funding to educational bureaucracies that have shown little ability to innovate or improve themselves. By year’s end, NSF had even gone to the extreme measure of rescinding funds for initiatives in the District of Columbia, Florida, and North Carolina. NSF cited a “crisis in leadership” in these school systems, but many see the systemic program as a leadership failure at NSF.

However, history ultimately judges the systemic initiatives, the net effect has been to virtually eliminate funding of innovative, independent programs. Furthermore, representatives from systemic initiatives have become the dominant voice in NSF panels that review prospective grant proposals. The tenor of current reviews is antipathetic to programs, such as ours, that deal with advanced topics or appeal to well-prepared teachers. Even so, we are among the few genetics education programs in the nation that has managed to maintain a reasonable level of federal funding during this difficult period.

**Corporate Advisory Board Is Key to Our Continued Success**

Five years ago, funding from the NSF and other federal agencies contributed nearly half of DNALC operating revenues. Federal funding dropped to 25% of 1996 costs. Thankfully, over the last several years, the DNALC has expanded funding from its local educational activities and from Long Island contributors to cover about half of annual operating costs. This strong base of local support allows us to weather periodic changes in federal funding and explore new grant opportunities. The Corporate Advisory Board (CAB) has become the key element of our local funding picture, involving an ever-growing number of local companies in the DNALC’s mission to educate local students. CAB members include individual philanthropists and business leaders representing a range of Long Island companies.

The year began with the good news that John Leahy had assumed the CAB chairmanship. John is Senior Vice President at Chase Manhattan Bank, a long-time supporter of CSHL. Under the leadership of John and CSHL Special Projects Coordinator Laura Hundt, the DNALC annual fund drive reached $147,000, a 10% increase over 1995. Horst Saalbach and golf committee mem-
bers organized the third annual golf tournament, held at Piping Rock Club on June 18th, which contributed $83,500 toward the annual fund goal. The golf committee included Vincent Adimando, Andrew Ackerman, Howard Blankmann, Gerald Brennan, G. Morgan Browne, Richard Catalano, Clark Gillies, Arthur Herman, John Kean, and William Keen. Long Island Business Night, on October 6th, was another highlight of the calendar as CAB members and guests were entertained by CSHL President James Watson and his wife Elizabeth at their home, Ballybung.

Opening of McClintock Exhibit Completes DNALC Renovation

On September 20th, we opened a new exhibit, "World of Barbara McClintock," to commemorate the life and work of Cold Spring Harbor Laboratory's most celebrated female scientist. The opening marked the completion of our long-term effort to remodel our 1925 school building as a modern science center.

The exhibit recreates Barbara's laboratory in the basement of Demerec Building at the time of her death in 1992. Since she was frugal and relied on little scientific equipment other than a microscope, her laboratory contained items that spanned her entire career at Cold Spring Harbor. Many articles date to her first laboratory in the Mouse House (now McClintock Laboratory), in which she worked until 1953. A timeline chronicles her childhood in Hartford and Brooklyn, her education at Cornell, and her scientific work at the California Institute of Technology and the University of Missouri, as well as Cold Spring Harbor. Her Nobel-winning discovery of transposable genetic elements is described in a computer animation. Her 1983 Nobel prize is displayed, along with her Nobel Lecture—one of the few videotapes of her available.

The McClintock gallery is painted to simulate the cinder block walls in the Demerec building laboratory in which she worked. The life model was commissioned from Third Dimension, Ltd., in Vancouver.
The most eye-catching, and controversial, part of the exhibit is a life model of Barbara, based on a photograph from 1947, six years after she came to Cold Spring Harbor as a summer guest. She is portrayed at work in front of her microscope, which was, in a real sense, her world: "You know, when I look at a cell, I get down in that cell and look around. I found that the more I worked with them, the bigger and bigger [the chromosomes] got. And when I was working with them, I wasn't outside, I was down there. I was part of the system. I was right down there and everything got big. I was even able to see the integral parts of the chromosome—actually, everything was there. It surprised me, because I actually felt as if I were right down there and these things were my friends."

Staff and Interns

In summer, we were sorry to say good-bye to part-time laboratory instructor Flavio Della Seta, who finished his postdoctoral stay in Kim Arndt's lab and returned to his faculty position at the University of Nancy. With the expansion of off-site instruction under the Genetics as a Model for Whole Learning Program, Diane Jedlicka and Malissa Hewitt had to cease instructing high school classes in the Bio2000 Laboratory. To fill this void, we were lucky to obtain the assistance of three additional part-time Laboratory Instructors—Joan Alexander, Scott Bronson, and Michael Greenberg. Joan, who has worked in several CSHL labs, is presently an associate scientist at Ampligen, a new biotechnology company concerned with identifying genes involved in human breast tumors. Scott is a research associate and Mike is a postdoctoral fellow in the laboratory of Jacek Skowronski, which investigates the role of the Nef protein in HIV pathogenesis.

Andrew Morotti joined the full-time staff in September as Laboratory Instructor, providing key support to the rapidly growing laboratory program. Andrew was a natural choice for the position, having worked all summer as an instructional intern for the Fun With DNA and World of Enzymes summer camps. In addition to teaching middle school labs, both at the DNALC and at participating schools, he also aided Mark Bloom in managing our growing number of high school interns. Andrew has a biology degree from SUNY Plattsburgh. Amy Cross began a part-time position as Program Assistant in August—helping with the complicated scheduling of visits to the DNALC and introducing students to the multimedia program Long Island Discovery and the Story of a Gene Exhibit. A 1996 graduate of SUNY Geneseo, Amy previously worked for Friends Academy in Locust Valley as a counselor and assistant office manager. Andrew and Amy also helped fill the large administrative hole left when Sue Lauter took maternity leave in September. Sue gave birth to twins, Casey and Martin, on November 14th.

Intern Trevor Carlson (Central Islip High School) contributed heavily to the development of the DNALC home page on the World Wide Web, including Shockwave animations and an interactive bulletin board. The laboratory instructional staff was ably assisted by high school interns Stacey Trotter (Walt Whitman High School), Salley Ann Gibney (Cold Spring Harbor High School), Rachael Neumann (Syosset High School), and Trevor Sammis (Huntington High School). Jermel Watkins, now the senior intern, continued to work part-time at the DNALC while studying pre-med at the New York Institute of Technology. In fall, we bid farewell to Stacey, who began her freshman year at Cornell University and to Salley, who
began her freshman year at Johns Hopkins University. Newcomers Hana Mizuno (Cold Spring Harbor High School), Mera Goodman (Walt Whitman High School), Karin Glaizer (Portledge School), Gerry DeGregoris (Chaminade High School), and Dan Gibson (Cold Spring Harbor High School) joined the staff in fall, 1996, and assisted with the laboratory instruction program. The new interns, together with Rachael and Trevor, began to formulate plans for carrying out independent research projects in addition to their intern work. Assisting at Fun With DNA summer camps were lab aides Ali Chaudry (Walt Whitman High School), Todd Rebori (Walt Whitman High School), Jennifer Kosinski (Roslyn High School), Paul Tanck (Massapequa High School), and Kim Bronson (who is married to Laboratory Instructor, Scott Bronson).

Staff Associates Jerry Watkins, of Central Islip High School, and Twana Adams, of Bronx Alternative School, instructed minority workshops hosted at the American Museum of Natural History in Manhattan and at A. Philip Randolph High School in Harlem. Jerry Watkins, father of DNALC intern Jermel, is a graduate of the DNA Science Workshop and the NSF Leadership Institute. In addition to instructing two DNA Science Workshops at the Museum of Natural History, Jerry also taught student workshops in his home district, Central Islip. A resident and community organizer, Twana taught Fun With DNA summer camps at A. Philip Randolph High School, with support from Michael Gordon, a teacher at Choir Academy of Harlem.

1996 Workshops, Meetings, and Collaborations

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<th>Date</th>
<th>Event</th>
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<tr>
<td>January 15-19</td>
<td>DNA Science Workshop, Walter and Eliza Hall Institute and University of Melbourne, Australia</td>
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<td>January 17</td>
<td>Laboratory for Women In Science and Engineering Program, SUNY Stony Brook, DNALC</td>
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<td>January 19</td>
<td>Corporate Advisory Board Meeting, Banbury Center</td>
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<td>February 10</td>
<td>Laboratory for Corporate Advisory Board and Cold Spring Harbor Laboratory Association Directors, DNALC</td>
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<td>February 23-25</td>
<td>National Science Foundation Workshop, Human Genome Diversity–Student Allele Database, USMA, West Point, New York</td>
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<td>March 2-3</td>
<td>National Science Foundation Follow-up Workshop, DNA Science, Bates College, Lewiston, Maine</td>
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<td>March 15-16</td>
<td>National Science Foundation Grant Review, Washington D.C.</td>
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<tr>
<td>March 16-17</td>
<td>National Science Foundation Follow-up Workshop, DNA Science, University of Kansas, Lawrence</td>
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<td>March 20</td>
<td>Meeting at American Philosophical Society, Philadelphia, Pennsylvania</td>
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<td>March 22</td>
<td>Benjamin/Cummings Strategies Workshop, Valencia Community College, Orlando, Florida</td>
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<td>March 28-29</td>
<td>National Science Teachers Association Meeting, Philadelphia, Pennsylvania</td>
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<td>March 30</td>
<td>Benjamin/Cummings Strategies Workshop, Pasadena Community College, California</td>
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<td>April 2</td>
<td>Meeting at The Center for Occupational Research and Development, Waco, Texas</td>
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<td>April 10</td>
<td>Community School District 29 Meeting, Rosedale, New York</td>
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<td>April 11</td>
<td>Site visit by Rob Kelly, Computer Associates, and Peter Goldsmith, Long Island Association</td>
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<td>April 17</td>
<td>Corporate Advisory Board Meeting, DNALC</td>
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<td>April 18</td>
<td>Great Moments in DNA Science, Honors Students Seminar, CSHL</td>
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<td>April 23</td>
<td>Great Moments In DNA Science, Honors Students Seminar, DNALC</td>
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<td>April 25</td>
<td>Biotech Conference, Harrisonburg High School, Harrisonburg, Virginia</td>
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<td>April 27-29</td>
<td>National Science Foundation Workshop, Human Genome Diversity–Student Allele Database, Howard University, Washington D.C.</td>
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<td>May 2</td>
<td>Site visit by BBC Photographers, Great Britain</td>
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<td>May 6</td>
<td>Great Moments in DNA Science, Honors Students Seminar, CSHL</td>
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<td>May 10</td>
<td>Site visit by Bruce Curtis, National Geographic</td>
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<td>May 15</td>
<td>Computer Associates Meeting, Islandia, New York</td>
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<td>May 20-21</td>
<td>Corporate Advisory Board Meeting, Chase Bank Regional Headquarters, Melville, New York</td>
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<td>May 28</td>
<td>Site visit by Ellen Potter, Salk Institute</td>
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<td>June 14</td>
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<td>June 17</td>
<td>National Science Foundation Workshop, DNA Science, University of Alaska, Fairbanks</td>
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<td>Access Excellence Summit, San Francisco, California</td>
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<td>June 24–July 2</td>
<td>Advanced DNA Science Workshop, DNALC</td>
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<td>Advanced DNA Science Minority Workshop, Central Islip, New York</td>
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<td>July 12</td>
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<td>July 15–19</td>
<td>National Science Foundation Workshop, DNA Science, Harrisonburg, Virginia</td>
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<td>Howard Hughes/Barker Welfare Foundation Minority Workshop, DNA Science, American Museum of Natural History, New York, New York</td>
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<td>July 22–26</td>
<td>DNA Science Workshop, DNALC</td>
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<td>Howard Hughes/Barker Welfare Foundation Minority Workshop, DNA Science, American Museum of Natural History, New York, New York</td>
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<td>July 29–Aug 1</td>
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<td>August 8</td>
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<td>August 12–15</td>
<td>Fun With DNA Workshop, DNALC</td>
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<td>August 19–23</td>
<td>DNA Science Workshop, DNALC</td>
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<td>August 26–29</td>
<td>Advanced DNA Science Workshop, Beckman Neuroscience Center, CSHL</td>
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<td>August 26–30</td>
<td>World of Enzymes Workshop, DNALC</td>
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<td>September 5</td>
<td>Site visit by Dorothy Dart and Ray Gesteland, University of Utah Genome Center</td>
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<td>September 17</td>
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<td>September 25</td>
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<td>October 7</td>
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<td>Seminar for Federal Judicial Center, DNALC and Banbury Center</td>
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<td>Site visit by Agneta Levinovitz, Nobel Forum</td>
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<td>October 18–19</td>
<td>National Association of Biology Teachers Convention, Charlotte, North Carolina</td>
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<td>October 21</td>
<td>Site visit by Dr. Nancy Douzines and Brooke Mahoney, The Rauch Foundation, and Linda Franciscovitch, US Trust Company</td>
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<td>October 22</td>
<td>Meeting at The Institute of Genomic Research, Rockville, Maryland</td>
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<td>October 23–25</td>
<td>Howard Hughes Medical Institute Program Directors' Meeting, Bethesda, Maryland</td>
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<td>October 26</td>
<td>Association of Science and Technology Centers Conference, Pittsburgh, Pennsylvania</td>
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<td>Oct 30–Nov 1</td>
<td>National Science Teachers Association Meeting, Atlanta, Georgia</td>
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<td>November 2</td>
<td>Seminar for Metropolitan Association of College and University Biologists, Paramus, New Jersey</td>
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<td>November 9</td>
<td>Laboratory for Journalists Workshop–Genetics of Human Behavior, DNALC</td>
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<td>November 9–11</td>
<td>National Science Foundation Workshop, Human Genome Diversity–Student Allele Database, Boston University, Massachusetts</td>
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<td>November 13</td>
<td>Seminar for Cornell University Medical College Program in Regional Genetics, CSHL</td>
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<td>November 14</td>
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<td>Site visit by Nancy Hutchison, Fred Hutchinson Cancer Center</td>
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### Sites of Major Faculty Workshops 1985–1996

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<th>College</th>
<th>Middle School</th>
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<td>ALABAMA</td>
<td>University of Alabama, Tuscaloosa</td>
<td>1987–1990</td>
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<td>ALASKA</td>
<td>University of Alaska, Fairbanks</td>
<td>1996</td>
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<td>ARIZONA</td>
<td>Tuba City High School</td>
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<td>ARKANSAS</td>
<td>Henderson State University, Arkadelphia</td>
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<td>CALIFORNIA</td>
<td>University of California, Davis</td>
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<td>San Francisco State University</td>
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<td>University of California, Northridge</td>
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<td>COLORADO</td>
<td>Colorado College, Colorado Springs</td>
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<td></td>
<td>United States Air Force Academy, Colorado Springs</td>
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<td>CONNECTICUT</td>
<td>Choate Rosemary Hall, Wallingford</td>
<td>1987</td>
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<td>DISTRICT OF COLUMBIA</td>
<td>Howard University</td>
<td>1992, 1996</td>
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<tr>
<td>FLORIDA</td>
<td>North Miami Beach Senior High School</td>
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<td>University of Western Florida, Pensacola</td>
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<td>Armwood Senior High School, Tampa</td>
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<td>GEORGIA</td>
<td>Fernbank Science Center, Atlanta</td>
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<td>Morehouse College, Atlanta</td>
<td>1991, 1996</td>
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<tr>
<td>HAWAII</td>
<td>Kamehameha Secondary School, Honolulu</td>
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<td>ILLINOIS</td>
<td>Argonne National Laboratory</td>
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<td>University of Chicago</td>
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<td>INDIANA</td>
<td>Butler University, Indianapolis</td>
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<td>IDAHO</td>
<td>University of Idaho, Moscow</td>
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<td>IOWA</td>
<td>Drake University, Des Moines</td>
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<tr>
<td>KANSAS</td>
<td>University of Kansas, Lawrence</td>
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<td>KENTUCKY</td>
<td>Murray State University</td>
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<td>Western Kentucky University, Bowling Green</td>
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<td>Jefferson Parish Public Schools, Harvey</td>
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<td>John McDonogh High School, New Orleans</td>
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<td>LOUISIANA</td>
<td>Bates College, Lewiston</td>
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<td>Annapolis Senior High School</td>
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<td>Frederick Cancer Research Center, Frederick</td>
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<td>McDonogh School, Baltimore</td>
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<td>Montgomery County Public Schools</td>
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<td>St. John’s College, Annapolis</td>
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<td>Beverly High School</td>
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<td>Dover-Sherborn High School, Dover</td>
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<td>Randolph High School</td>
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<td>Winsor School, Boston</td>
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<td>MASSACHUSETTS</td>
<td>Boston University</td>
<td>1994, 1996</td>
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<td>Athens High School, Troy</td>
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<td>Mississippi School for Math &amp; Science, Columbus</td>
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<td>Washington University, St. Louis</td>
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<td>St. Paul’s School, Concord</td>
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<td>University of Nevada, Reno</td>
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<td>Albany High School</td>
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<td>Bronx High School of Science</td>
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<td>Columbia University, New York</td>
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Cold Spring Harbor High School 1985, 1987
DeWitt Middle School, Ithaca 1991, 1993
DNA Learning Center 1988–1995
**DNA Learning Center** 1990, 1992, 1995
DNA Learning Center 1990–1992
Fostertown School, Newburgh 1991
Huntington High School 1986
Irvington High School 1986
Junior High School 263, Brooklyn 1991
Lindenhurst Junior High School 1991
Orchard Park Junior High School 1991
Plainview-Old Bethpage Middle School 1991
State University of New York, Purchase 1989
State University of New York, Stony Brook 1987–1990
Titusville Middle School, Poughkeepsie 1991, 1993
Wheatley School, Old Westbury 1985
**U.S. Military Academy, West Point** 1996

**NORTH CAROLINA**
North Carolina School of Science, Durham 1987
OHIO
Case Western Reserve University, Cleveland 1990
Cleveland Clinic 1987
North Westerville High School 1990

**OKLAHOMA**
School of Science and Mathematics, Oklahoma City 1994

**PENNSYLVANIA**
Duquesne University, Pittsburgh 1988
Germantown Academy 1988

**SOUTH CAROLINA**
Medical University of South Carolina, Charleston 1988
University of South Carolina, Columbia 1988

**TEXAS**
J.J. Pearce High School, Richardson 1990
Langham Creek High School, Houston 1991
Tift High School, San Antonio 1991
**Trinity University, San Antonio** 1994

**UTAH**
University of Utah, Salt Lake City 1993

**VERMONT**
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**VIRGINIA**
Eastern Mennonite University, Harrisonburg 1996
Jefferson School of Science, Alexandria 1987
Mathematics and Science Center, Richmond 1990

**WASHINGTON**
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**WEST VIRGINIA**
Bethany College 1989

**WISCONSIN**
Marquette University, Milwaukee 1986, 1987
University of Wisconsin, Madison 1988, 1989

**WYOMING**
University of Wyoming, Laramie 1991

**AUSTRALIA**
Walter and Eliza Hall Institute and University of Melbourne 1996

**CANADA**
Red River Community College, Winnipeg, Manitoba 1989

**ITALY**
International Institute of Genetics and Biophysics, Naples 1996

**PANAMA**
University of Panama, Panama City 1994

**PUERTO RICO**
University of Puerto Rico, Mayaguez 1992
**University of Puerto Rico, Mayaguez** 1992
**University of Puerto Rico, Rio Piedras** 1993
University of Pureto Rico, Rio Piedras 1994

**RUSSIA**
Shemyakin Institute of Bioorganic Chemistry, Moscow 1991

**SWEDEN**
Kristineberg Marine Research Station, Fiskebäckskil 1995
1996 PUBLICATIONS

General Books
Protein Kinesis: The Dynamics of Protein Trafficking and Stability
Symposia on Quantitative Biology LX
Vaccines 96: Molecular Approaches to the Control of Infectious Diseases
F. Brown, E. Norrby, D. Burton, and J. Mekalanos (eds.)
The Eighth Day of Creation: Makers of the Revolution in Biology, Expanded Edition
H.F. Judson
Discovering Molecular Genetics: Solutions Manual & Workbook
J.H. Miller

CSHL Monograph Series
DNA Replication in Eukaryotic Cells
M.L. DePamphilis (ed.)
Epigenetic Mechanisms of Gene Regulation
V.E.A. Russo, R.A. Martienssen, and A.D. Riggs (eds.)

Cancer Surveys Series
Vol. 26: Skin Cancer
I.M. Leigh, J.A. Newton Bishop, and M.L. Kripke (eds.)
Vol. 27: Cell Signalling
P. Parker and T. Pawson (eds.)
Vol. 28: Genetic Instability in Cancer
T. Lindahl (ed.)

Journals
Genes & Development (Volume 10, 24 issues)
T. Grodzicker and N. Hastie (eds.)
Genome Research (Volume 6, 12 issues)
A. Chakravarti, R. Gibbs, E. Green, R. Myers, and M. Boguski (eds.)
Learning & Memory (Volume 3, 6 issues)
J.H. Byrne (ed.)

Videotapes
On Becoming a Scientist
Promise & Perils of Biotechnology: Genetic Testing

Other
The Lab Manual Source Book
CSHL Annual Report 1995
Banbury Center Annual Report 1995
Administration and Financial Annual Report 1995
Abstract/program books for 16 CSHL meetings
COLD SPRING HARBOR LABORATORY PRESS

The year was one of considerable achievement in the creation of assets and sales of existing publications. All three journals built a stronger subscription base. A new edition of The Cold Spring Harbor Laboratory Manual Source Book was published and by the year's end had become the basis of a new company formed to capitalize on the potential of the directory and its online companion, BioSupplyNet. Nine new books and two new videotapes were published, bringing the total titles in print to over 220. However, publication of six new book titles, including the most important of the year, could not be completed by year's end and despite the backlist's strong performance there was a considerable shortfall in book sales. Journal income reached a record total overall but soft advertising sales in the first half-year and higher costs resulting from staff disabilities reduced the anticipated surplus. The Press thus showed an overall operating loss. A comprehensive response to the factors underlying this result was already underway by the year's end.

Books and Electronic Media

No new laboratory manuals were published this year, as work continued on the development of a bumper crop of new techniques publications for future release. Of the eleven books and videotapes published during the year (see opposite for the complete list), two titles were added to the monograph series that has brought distinction to our program since the early 1970s. DNA Replication in Eukaryotic Cells, orchestrated singlehandedly by editor Mel DePamphilis, had three goals: to help teachers of molecular biology synthesize the enormous amount of new information available on the topic; to provide workers in the field with a summary of progress; and to help other investigators relate DNA replication to other biological problems such as cell cycle control and cell death. Its forty chapters divided neatly into concepts, proteins and systems, and we anticipate future editions in which selected parts of this material are made available for teachers and students. Epigenetic Mechanisms of Gene Replication, edited by Enzo Russo, Rob Martienssen, and Arthur Riggs, was dedicated to Barbara McClintock, forever associated with Cold Spring Harbor, where she discovered transposable genetic elements. By the end of her long life, she had seen the study of epigenetic mechanisms she pioneered move from the shadows of uncertainty to the status of a respected discipline. The book's 35 chapters provided, for the first time, a survey of such mechanisms in mammalian, plant, insect, fungal, and microbial systems.

It also gave us much satisfaction to release in a new edition Horace Freeland Judson's classic history of the birth of molecular biology, The Eighth Day of Creation. First published in 1978 and unavailable in the U.S. for many years, it is one of the best popular accounts of any aspect of science ever published, the product of Judson's graceful writing and his journalist's tenacity in getting to the heart of a great story. Our handsomely designed new edition corrects some minor errors in the original and extends it with additional essays on Rosalind Franklin and Erwin Chargaff and a new epilogue on the development of molecular biology since 1970. The book's reappearance has been warmly welcomed.

It is also worth noting the appearance of, perhaps, the last two in a series of educational videotapes conceived by staff at the University of California San Francisco. On Becoming A Scientist, The Promise and Perils of Biotechnology, and last year's Stories from the Scientists were produced by a group of Bay Area teachers and university staff as a follow-up to UCSF's hugely popular 1992 symposium on the social implications of molecular genetics, Winding Your Way Through DNA, which the Press also distributed on videotape. Created with care and clarity, and showing science and scientists in action in a truthful way, all these productions have been popular with educators at many levels and have been recognized with awards, such as this year's Golden
Eagle and Golden Apple, won by *Stories from the Scientists*, and the Bronze Apple won by *Promise and Perils*. The coordinator of the series at UCSF throughout our collaboration was Valli Thayer McDougle, now retired from her position, with whom it was always a pleasure to work and to whom the lion's share of credit for this inspiring series must go.

Our history, *The Cells of the Body*, written by Sir Henry Harris, was selected by CHOICE magazine, a publication of the Association of College and Research Libraries, for its list of Outstanding Academic Books for 1996.

**Journal Publishing**

For the first time in several years, the journal department had none of the challenges of a new title launch or a doubled publication frequency to deal with. But significant progress was made towards electronic distribution of journal content, starting in March with the addition of abstracts from all the current issues to the journals' World Wide Web pages. By year's end, agreement had been reached with HighWire Press of Stanford University to publish the full text of all the Cold Spring Harbor journals online, starting in late 1997. HighWire's list of client publishers now encompasses many of the most important journals in cellular and molecular biology and their clustering under one virtual roof gives added point to many interesting questions about the future of journals in a wired world.

The geographically far-flung editors of *Genome Research* took enthusiastic advantage of another form of networked information, namely a journal-specific, web-based intranet used to inspect and comment on submitted papers and their reviews. This prototype system, devised by Managing Editor Judy Cuddihy and the staff of Sheridan Electronic Systems, proved robust and scalable and will be adapted for use with our other journals, saving time and the expense of sending papers for review around the world by courier.

*Genome Research* ended its first full year of monthly publication with a healthy 12% increase in institutional subscriptions, more than justifying the decision to reposition our established PCR technology journal in genome science. The journal's manuscript submission rate grew steadily throughout the year and the journal's editors, Mark Boguski, Aravinda Chakravarti, Richard Gibbs, Eric Green, and Richard Myers, responded bravely to the increased workload and the need for constantly advancing standards. By the end of the year, however, all concerned agreed that it was time the journal had a full-time editor at Cold Spring Harbor to help make the selection process more efficient and to create additional types of editorial content that would enhance the journal's appeal to individual subscribers.

*Learning and Memory*, a new addition to our journal family in mid-1994, maintained its subscription base in its second full year of publication. The flow of manuscripts strengthened, and the year was notable for the appearance of an outstanding double issue on longterm potentiation and its meaning. The journal is steadily achieving recognition in the neuroscience community, a difficult process given the competition from more established journals and the fledgling stage of much molecular analysis of learning and memory. Its future is in the capable hands of Jack Byrne, who took over as editor this year. The journal has a niche and a foundation for eventual success, but it will not come overnight.

*Genes & Development*, on the other hand, is now an established winner. Its reputation was underlined by a further increase in the impact factor calculated by the Institute for Scientific Information, which placed it sixth in the roster of most cited journals in all biology. The journal's editors, Terri Grodzicker and Nick Hastie, facing increased demand to publish in the journal, responded by continuing to raise the standard required for acceptance. The year's 24 issues stretched the scope of the journal into newer areas of cellular biology, and the activities of Michele Cleary, and later Kim Gavin, resulted in the appearance of more commissioned reviews. The reward, with the aid of well-targeted marketing, was a 5% increase in circulation—no small achievement for a journal in its
Advertising sales were soft in the first half of the year but picked up well in the third quarter with the arrival of Marcie Ebenstein as Advertising Sales Manager. She began an overhaul of the sales department’s operations in order to rebuild this important source of income for the journal program.

The Laboratory Manual SourceBook

In March, the 1996 edition of this directory of laboratory products and their suppliers was distributed free of charge to more than 50,000 scientists worldwide. The cost of distribution was borne by the product advertising space, sold vigorously by Joan Boyce, the project manager. This was the second annual edition of the SourceBook, more comprehensive in all respects than the first. The expanded databases were incorporated within BioSupplyNet, a searchable web site that extended the information in the print directory with technical specifications, special pricing offers, and other user-oriented data. The SourceBook and its interactive equivalent grew from the laboratory manual publishing program and, as they evolved, the project’s potential basis for systems of advanced marketing and electronic commerce seemed ever more intriguing. The development of these capabilities, however, clearly required technical expertise that we did not have, and an investment that the Press could not sustain. Fortunately, we did have access to the Harris and Harris Group, a Manhattan-based business development company wise in the commercial exploitation of ideas and technology from academic institutions. With the help of its principals, a new company was formed in October to purchase and develop the SourceBook and its web site. Owned jointly by the Laboratory and its investors, BioSupplyNet Incorporated has an experienced entrepreneur, Lyle Brecht, as its President, Joan Boyce as Vice-President, and the SourceBook’s ex-Press core staff as its first employees.

The company’s first challenge was to create a 1997 print directory so comprehensive that it would become the standard for science and its associated vendors. The second was to incorporate into the web site the technical sophistication necessary to surge ahead of competitors in search capability and delivery of advanced marketing information. As the year ended, the staff had risen to eight, and both tasks were well underway. A joint marketing agreement with the Press was negotiated by which all purchasers of Cold Spring Harbor manuals would continue to get free copies of the SourceBook.

Marketing and Distribution

This year’s marketing activities centered on the well-tried strategies of direct mail, meeting exhibits, and journal advertising, but incorporated additional activities on an exploratory basis. The direct mail program centered on a widely distributed Genes, Cells, and Proteins catalog, two issues of the New Book Titles newsletter, and a variety of title-specific brochures. The journals received particular attention through a well-targeted approach to specific academic libraries and reduced price offers to individuals via several different routes. Both strategies had notable success. In addition, some telemarketing was done to obtain specific types of information.

The Press exhibit stand appeared at the seven major scientific society meetings of the year and several smaller, more specific conferences. This is another important marketing channel which introduces the Press to many young scientists and to hopeful authors and others with ideas for future publications.

The Cold Spring Harbor meetings and courses offer similar opportunities in more intimate surroundings. The campus bookstore, in the past operated by the Press in cramped surroundings, reopened in April in new and spacious quarters under the management of the college division of Barnes and Noble, Inc. Visitors benefited from a much expanded range of books, software, clothing, and other items, with room and expanded hours in which to browse.

The web site at www.cshl.org, which opened two years ago as a marketing tool for the Press and the meetings and courses program, was redesigned to reflect other aspects of the Laboratory. Its function for the Press remained unchanged—a keyword-searchable catalog allied with detailed
announcements of new books and journal issues, sample chapters from forthcoming titles, and the opportunity to purchase electronically. But an important shift in emphasis occurred this year with our decision not to release a complete catalog of publications in print. The online catalog is now definitive, to be recommended to any customers seeking backlist information, and much care is taken to ensure that it is complete, up-to-date, and entirely accurate in all respects.

To service book orders and journal subscriptions acquired by these various means, our Fulfillment and Dispatch Department continued to rely on its now drastically outmoded computer system while actively seeking an alternative with the kind of functionality needed by an operation of our size. Towards the year’s end, one promising software prototype did well in early tests, suggesting that it might be possible early in 1997 to install a new client-server based system that would offer unprecedented flexibility and control together with the kind of financial and marketing analysis on the fly that is impossible with the current equipment.

New Projects

During the year, agreement was reached with authors and editors about the creation of more than 20 new publishing projects, in print and in electronic media. These projects included manuals, monographs, advanced textbooks, and essays. And many conversations took place about other possibilities in a variety of media, as publishers, scientists, and others pooled their ideas about likely styles of scientific communication in the next century.

Staff changes

The publishing program continued to benefit from the commitment of its staff to high quality at every level and in every function. The list of staff members in December 1996 is given at the back of this volume, and grateful thanks is extended to each one of them. But special mention must be made of the heads of the departments of the Press, Nancy Ford, Judy Cuddihy, Ingrid Benirschke, Nancy Hodson, and Guy Keyes, whose management skills, ideas, and advice were essential to the workings of the organization.

During the year, we welcomed several new members of staff: Marcie Ebenstein as Advertising Sales Manager, Kim Gavin as Assistant Editor of Genes & Development, Kaaren Janssen as Project Managing Editor, Val Pakaluk as Editorial Secretary, and Liz Powers as Assistant to the Director. We bade farewell to Michele Cleary, Eileen Paetz, Laura Terwilliger, and Teresa Tiganis, wishing them well for the future. And we prepared to say goodbye to Nancy Ford who in December announced her intention to retire in January, after 24 years' distinguished service to the Laboratory. During this period she developed the publications program with taste, intelligence, and dedication, working with an extraordinary cadre of leading scientists to create some of the landmark publications in the nascent science of molecular biology.

Nancy built into the publishing program many of the elements we value most: insistent accuracy, elegant design, and warm relationships with the authors and editors who bring us their special expertise. Those values remain intact, now the preserve of others with equal commitment—a legacy to be very proud of. We began the new year with the intention of restructuring the organization of the Press to increase efficiency, raise productivity, and improve financial management. Our challenge is to attain these goals without losing sight of the characteristics that have defined publishing at Cold Spring Harbor for the past quarter century.

John R. Inglis
## FINANCIAL STATEMENTS

### STATEMENT OF FINANCIAL POSITION
December 31, 1996
With comparative amounts for 1995

<table>
<thead>
<tr>
<th>Assets:</th>
<th>1996</th>
<th>1995</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash and cash equivalents</td>
<td>$12,959,798</td>
<td>13,459,548</td>
</tr>
<tr>
<td>Investments</td>
<td>123,682,720</td>
<td>94,979,330</td>
</tr>
<tr>
<td>Accounts receivable:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Publications</td>
<td>489,932</td>
<td>601,297</td>
</tr>
<tr>
<td>Other</td>
<td>191,162</td>
<td>358,430</td>
</tr>
<tr>
<td>Grants receivable</td>
<td>2,251,645</td>
<td>3,028,747</td>
</tr>
<tr>
<td>Contributions receivable</td>
<td>3,518,165</td>
<td>500,000</td>
</tr>
<tr>
<td>Publications inventory</td>
<td>1,426,118</td>
<td>1,394,720</td>
</tr>
<tr>
<td>Prepaid expenses and other assets</td>
<td>1,308,461</td>
<td>2,078,234</td>
</tr>
<tr>
<td>Investment in employee residences</td>
<td>2,370,943</td>
<td>1,825,641</td>
</tr>
<tr>
<td>Land, buildings, and equipment:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Land and improvements</td>
<td>9,037,008</td>
<td>7,588,532</td>
</tr>
<tr>
<td>Buildings</td>
<td>60,467,671</td>
<td>59,706,386</td>
</tr>
<tr>
<td>Furniture, fixtures, and equipment</td>
<td>4,310,386</td>
<td>4,239,740</td>
</tr>
<tr>
<td>Laboratory equipment</td>
<td>11,721,056</td>
<td>10,554,994</td>
</tr>
<tr>
<td>Library books and periodicals</td>
<td>365,630</td>
<td>365,630</td>
</tr>
<tr>
<td>Construction in progress</td>
<td>1,103,850</td>
<td>190,382</td>
</tr>
<tr>
<td></td>
<td>87,005,601</td>
<td>82,645,664</td>
</tr>
<tr>
<td>Less accumulated depreciation and amortization</td>
<td>(27,354,072)</td>
<td>(24,634,265)</td>
</tr>
<tr>
<td>Land, buildings, and equipment, net</td>
<td>59,651,529</td>
<td>58,011,399</td>
</tr>
</tbody>
</table>

Total assets $207,850,473 176,237,346

<table>
<thead>
<tr>
<th>Liabilities and Net Assets:</th>
<th>1996</th>
<th>1995</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accounts payable and accrued expenses</td>
<td>$1,470,007</td>
<td>949,079</td>
</tr>
<tr>
<td>Notes payable</td>
<td>864,961</td>
<td>667,520</td>
</tr>
<tr>
<td>Bonds payable</td>
<td>30,000,000</td>
<td>30,000,000</td>
</tr>
<tr>
<td>Deferred revenue</td>
<td>5,715,510</td>
<td>5,351,669</td>
</tr>
<tr>
<td>Total liabilities</td>
<td>38,050,478</td>
<td>36,968,268</td>
</tr>
</tbody>
</table>

Net Assets:

Unrestricted

| General operating | 6,030,181 | 5,693,880 |
| Designated: | | |
| For research program | 1,150,000 | 1,150,000 |
| Capital expenditures | 34,540,515 | 34,010,961 |
| Board designated endowment | 63,334,506 | 49,544,118 |
| Total unrestricted | 105,055,202 | 90,398,959 |
| Temporarily restricted | 1,269,123 | 470,000 |
| Permanently restricted | 63,475,670 | 48,400,119 |
| Total net assets | 169,799,995 | 139,269,078 |
| Total liabilities and net assets | $207,850,473 | 176,237,346 |
## STATEMENT OF ACTIVITIES
### Year Ended December 31, 1996
#### With comparative amounts for 1995

<table>
<thead>
<tr>
<th>Revenue and other support:</th>
<th>Unrestricted</th>
<th>Temporarily Restricted</th>
<th>Permanently Restricted</th>
<th>1996 Total</th>
<th>1995 Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Public support (contributions and nongovernment grant awards)</td>
<td>$9,639,448</td>
<td>1,179,123</td>
<td>3,537,198</td>
<td>14,355,769</td>
<td>11,191,359</td>
</tr>
<tr>
<td>Government grant awards</td>
<td>13,756,042</td>
<td>–</td>
<td>–</td>
<td>13,756,042</td>
<td>12,105,861</td>
</tr>
<tr>
<td>Indirect cost allowances</td>
<td>9,817,300</td>
<td>–</td>
<td>–</td>
<td>9,817,300</td>
<td>9,007,870</td>
</tr>
<tr>
<td><strong>Other revenue:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Program fees</td>
<td>1,948,793</td>
<td>–</td>
<td>–</td>
<td>1,948,793</td>
<td>1,773,800</td>
</tr>
<tr>
<td>Rental income</td>
<td>443,934</td>
<td>–</td>
<td>–</td>
<td>443,934</td>
<td>435,555</td>
</tr>
<tr>
<td>Publications sales</td>
<td>4,804,744</td>
<td>–</td>
<td>–</td>
<td>4,804,744</td>
<td>5,119,484</td>
</tr>
<tr>
<td>Dining services</td>
<td>2,080,803</td>
<td>–</td>
<td>–</td>
<td>2,080,803</td>
<td>1,983,219</td>
</tr>
<tr>
<td>Rooms and apartments</td>
<td>1,639,324</td>
<td>–</td>
<td>–</td>
<td>1,639,324</td>
<td>1,664,609</td>
</tr>
<tr>
<td>Royalty &amp; licensing fees</td>
<td>464,652</td>
<td>–</td>
<td>–</td>
<td>464,652</td>
<td>1,222,390</td>
</tr>
<tr>
<td>Gain on sale of investments, net</td>
<td>5,911,392</td>
<td>–</td>
<td>–</td>
<td>14,682,742</td>
<td>4,410,605</td>
</tr>
<tr>
<td>Net decrease in unrealized gains on investments</td>
<td>(1,985,032)</td>
<td>–</td>
<td>–</td>
<td>(5,535,803)</td>
<td>–</td>
</tr>
<tr>
<td>Investment income - interest and dividends</td>
<td>6,258,338</td>
<td>–</td>
<td>–</td>
<td>6,258,338</td>
<td>6,903,504</td>
</tr>
<tr>
<td>Recovery of valuation allowance</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>522,774</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>109,733</td>
<td>–</td>
<td>–</td>
<td>109,733</td>
<td>134,334</td>
</tr>
<tr>
<td><strong>Total other revenue</strong></td>
<td>21,676,681</td>
<td>–</td>
<td>5,220,579</td>
<td>26,897,260</td>
<td>24,170,274</td>
</tr>
<tr>
<td>Net assets released from restrictions:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expiration of time restrictions</td>
<td>380,000</td>
<td>(380,000)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Total revenue and support</strong></td>
<td>55,269,471</td>
<td>799,123</td>
<td>8,757,777</td>
<td>64,826,371</td>
<td>56,475,354</td>
</tr>
</tbody>
</table>

| Expenses: | | | | | |
| Program services: | | | | | |
| Research | 16,658,074 | – | – | 16,658,074 | 15,837,311 |
| Summer programs (meetings and courses) | 5,730,064 | – | – | 5,730,064 | 5,201,484 |
| Publications | 5,031,983 | – | – | 5,031,983 | 5,078,559 |
| Banbury Center conferences | 457,033 | – | – | 457,033 | 920,781 |
| DNA Learning Center programs | 278,473 | – | – | 278,473 | 486,367 |
| **Total program services** | 28,155,627 | – | – | 28,155,627 | 27,524,502 |
| Supporting services: | | | | | |
| Direct research support | 1,325,133 | – | – | 1,325,133 | 1,243,713 |
| Library | 658,867 | – | – | 658,867 | 591,520 |
| Operation and maintenance of plant | 5,721,030 | – | – | 5,721,030 | 5,514,513 |
| General and administrative | 4,824,906 | – | – | 4,824,906 | 4,655,785 |
| Dining services | 1,907,328 | – | – | 1,907,328 | 1,814,174 |
| Interest | 1,385,706 | – | – | 1,385,706 | 1,526,387 |
| Depreciation and amortization | 2,988,112 | – | – | 2,988,112 | 2,820,555 |
| **Total supporting services** | 18,811,182 | – | – | 18,811,182 | 18,166,647 |
| **Total expenses** | 46,966,809 | – | – | 46,966,809 | 45,691,149 |
| **Change in net assets before cumulative effects of changes in accounting principles** | $8,302,662 | 799,123 | 8,757,777 | 17,859,562 | 10,784,205 |
| Cumulative effects of changes in accounting for investments and contributions | 6,353,581 | – | 6,317,774 | 12,671,355 | 1,560,000 |
| Change in net assets | 14,656,243 | 799,123 | 15,075,551 | 30,530,917 | 12,344,205 |
| **Net assets at beginning of year** | 90,398,959 | 470,000 | 48,400,119 | 139,269,078 | 126,924,873 |
| **Net assets at end of year** | $105,055,202 | 1,269,123 | 63,475,670 | 169,799,995 | 139,269,078 |
# COMPARATIVE OPERATING HISTORY 1992-1996
(Dollars in Thousands)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Income:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main Lab:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grants &amp; contracts</td>
<td>$16,800</td>
<td>18,136</td>
<td>19,293</td>
<td>19,653</td>
<td>20,879</td>
</tr>
<tr>
<td>Indirect cost reimbursement</td>
<td>8,388</td>
<td>8,383</td>
<td>8,460</td>
<td>8,881</td>
<td>9,704</td>
</tr>
<tr>
<td>Other</td>
<td>5,520</td>
<td>6,049</td>
<td>6,808</td>
<td>7,461</td>
<td>7,859</td>
</tr>
<tr>
<td>CSH Press</td>
<td>3,709</td>
<td>4,319</td>
<td>4,390</td>
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The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.

(1) Funds designated to underwrite future direct and indirect expenses of the neuroscience and other research programs.

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### COLD SPRING HARBOR LABORATORY
**SOURCES OF REVENUE**
**YEAR ENDED DECEMBER 31, 1996**

![Pie chart showing sources of revenue: Federal Grants 37.7%, Endowments and Board Designated Funds 12.5%, Foundation and Private Contributions and Grants 23.6%, Royalty and Licensing Fees 2.5%, Interest and Miscellaneous 1.4%, Corporate Contributions and Grants 3.9%, Auxiliary Activities 18.4%]
FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the DNA Learning Center receive a substantial portion of their funding through grants from the Federal Government and through grants, capital gifts, and annual contributions from private foundations, corporations, and individuals. The following section summarizes funding that occurred during 1996.

GRANTS January 1, 1996–December 31, 1996
COLD SPRING HARBOR LABORATORY

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* Equipment

* New Grants Awarded in 1996

+ Includes direct and indirect cost
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* New Grants Awarded in 1996
* Includes direct and indirect cost
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| Ira DeCamp Foundation                          | Equipment                      | 7/94 - 6/97 | 300,000 *
| Estate of Mildred Becker                       | Equipment                      | 6/96 - 5/97 | 100,000 * |
| Oliver S. and Jennie R. Donaldson Charitable Trust | Equipment                  | 12/96 - 11/97 | 100,000 * |
| Edwin Marks                                    | Equipment                      | 6/96 - 5/97 | 65,760 * |
| William and Maude Pritchard Charitable Trust    | Equipment                      | 12/96 - 11/96 | 225,000 * |
| Estate of H. Turner Slocum                     | Equipment                      | 3/96 - 2/97 | 5,000 * |

* New Grants Awarded in 1996
+ Includes direct and indirect cost
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<tr>
<td>Bayer Corporation</td>
<td>Hepatitis B Virus Meeting</td>
<td>1/96 - 12/96</td>
<td>1,008*</td>
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<tr>
<td>Bayer Corporation</td>
<td>Molecular Chaperones and the Heat-shock Response Meeting</td>
<td>1/96 - 12/96</td>
<td>2,000*</td>
</tr>
<tr>
<td>Beckman Instruments, Inc.</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
<td>10/96 - 9/97</td>
<td>5,000*</td>
</tr>
<tr>
<td>Behringerwerke AG, Marburg/Germany-Beohringer, Ingelheim GmbH, Ingelheim/Germany</td>
<td>Hepatitis B Virus Meeting</td>
<td>1/96 - 12/96</td>
<td>1,954*</td>
</tr>
<tr>
<td>Churchill Livingstone</td>
<td>Molecular Chaperones and the Heat-shock Response Meeting</td>
<td>1/96 - 12/96</td>
<td>500*</td>
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<tr>
<td>Clontech Laboratories, Inc.</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
<td>10/96 - 9/97</td>
<td>1,000*</td>
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<tr>
<td>Dupont Merck Pharmaceutical Company</td>
<td>61st Symposium: Function and Dysfunction in the Nervous System</td>
<td>3/96 - 12/96</td>
<td>1,000*</td>
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<tr>
<td>GenHunter Corporation</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
<td>10/96 - 9/97</td>
<td>2,000*</td>
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<tr>
<td>Genomyx Corporation</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
<td>10/96 - 9/97</td>
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<td>Glaxo Wellcome, Inc.</td>
<td>Hepatitis B Virus Meeting</td>
<td>1/96 - 12/96</td>
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<td>Hoffman-LaRoche Laboratories</td>
<td>Hepatitis B Virus Meeting</td>
<td>1/96 - 12/96</td>
<td>1,575*</td>
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<tr>
<td>F. Hoffman-LaRoche</td>
<td>Molecular Chaperones and the Heat-shock Response Meeting</td>
<td>1/96 - 12/96</td>
<td>500*</td>
</tr>
<tr>
<td>Heidie Meisendothen</td>
<td>Perspectives on the Zebrafish Genome</td>
<td>4/96 - 3/97</td>
<td>100*</td>
</tr>
<tr>
<td>ICN Pharmaceuticals, Inc.</td>
<td>Translational Control Meeting</td>
<td>1/96 - 12/96</td>
<td>2,000*</td>
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<tr>
<td>Merck &amp; Company, Inc.</td>
<td>Hepatitis B Virus Meeting</td>
<td>1/96 - 12/96</td>
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<tr>
<td>Merck Research Laboratories</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
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<tr>
<td>Micro Video Instruments, Inc.</td>
<td>Perspectives on the Zebrafish Genome</td>
<td>4/96 - 3/97</td>
<td>300*</td>
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<td>Millennium Pharmaceuticals</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
<td>10/96 - 9/97</td>
<td>1,500*</td>
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<td>Novagen, Inc.</td>
<td>Translational Control Meeting</td>
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<td>500*</td>
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<td>Novo Nordisk A/S</td>
<td>Molecular Chaperones and the Heat-shock Response Meeting</td>
<td>1/96 - 12/96</td>
<td>3,000*</td>
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<tr>
<td>Oxnard Foundation</td>
<td>Utrophin Meeting</td>
<td>11/96 - 10/99</td>
<td>20,000*</td>
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</tbody>
</table>

* New Grants Awarded in 1996
+ Includes direct and indirect cost
<table>
<thead>
<tr>
<th>Grantor</th>
<th>Program/Principal Investigator</th>
<th>Duration of Grant</th>
<th>1996 Funding*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacia Biotech</td>
<td>Molecular Approaches to the Control of Infectious Diseases</td>
<td>4/96 - 3/97</td>
<td>12,000 *</td>
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<td>Promega Corporation</td>
<td>Translational Control Meeting</td>
<td>1/96 - 12/96</td>
<td>1,500 *</td>
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<td>Qiagen, Inc.</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
<td>10/96 - 9/97</td>
<td>2,000 *</td>
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<td>Ribogene, Inc.</td>
<td>Translational Control Meeting</td>
<td>1/96 - 12/96</td>
<td>10,000 *</td>
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<td>Schering Plough Research</td>
<td>Hepatitis B Virus Meeting</td>
<td>1/96 - 12/96</td>
<td>2,000 *</td>
</tr>
<tr>
<td>SmithKline Beecham Pharmaceuticals</td>
<td>Differential Display and Related Techniques for Gene Discovery</td>
<td>10/96 - 9/97</td>
<td>1,000 *</td>
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<tr>
<td>Society for Developmental Biology</td>
<td>Perspectives on the Zebrafish Genome</td>
<td>4/96 - 3/97</td>
<td>2,500 *</td>
</tr>
<tr>
<td>StressGen Biotechnologies Corporation</td>
<td>Molecular Chaperones and the Heat-shock Response Meeting</td>
<td>1/96 - 12/96</td>
<td>5,000 *</td>
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<td>The Company of Biologists Limited</td>
<td>Perspectives on the Zebrafish Genome</td>
<td>4/96 - 3/97</td>
<td>4,669 *</td>
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**BANBURY CENTER**

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<thead>
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<th>Grantor</th>
<th>Program/Principal Investigator</th>
<th>Duration of Grant</th>
<th>1996 Funding*</th>
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<tr>
<td><strong>FEDERAL SUPPORT</strong></td>
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<tr>
<td>The Federal Judicial Center</td>
<td>Seminar for State and Federal Judges</td>
<td>1996</td>
<td>25,239 *</td>
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<tr>
<td><strong>NONFEDERAL SUPPORT</strong></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Meeting Support</strong></td>
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<td>Charles A. Dana Foundation</td>
<td>Research on the Genetic Basis of Manic Depressive Illness</td>
<td>1996</td>
<td>23,000 *</td>
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<tr>
<td>Geron Corporation</td>
<td>Telomeres</td>
<td>1996</td>
<td>1,500 *</td>
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<tr>
<td>Hereditary Diseases Foundation</td>
<td>Triple Repeats and Polyglutamine Tracts</td>
<td>1996</td>
<td>10,000 *</td>
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<tr>
<td>Osiris Therapeutics, Inc.</td>
<td>Mesenchyme</td>
<td>1996</td>
<td>31,999 *</td>
</tr>
<tr>
<td>Marie H. Robertson Memorial Fund for Neurobiology</td>
<td>Learning and Memory</td>
<td>1996</td>
<td>9,920 *</td>
</tr>
<tr>
<td>Albert B. Sabin Vaccine Foundation</td>
<td>Sabin HIV</td>
<td>1996</td>
<td>35,249 *</td>
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* New Grants Awarded in 1996
+ Includes direct and indirect cost
## DNA LEARNING CENTER

<table>
<thead>
<tr>
<th>Grantor</th>
<th>Program/Principal Investigator</th>
<th>Duration of Grant</th>
<th>1996 Funding*</th>
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<td><strong>FEDERAL GRANTS</strong></td>
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<tr>
<td><strong>NATIONAL SCIENCE FOUNDATION</strong></td>
<td>A Two-Part Program to Develop and Support Nationwide Corps of Human and Molecular Genetics Resource Teachers at the Secondary Level, David Micklos</td>
<td>4/93 - 6/97</td>
<td>124,417</td>
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<td></td>
<td>A Novel Mechanism for Introducing Human Genome Research in Freshman Biology, Mark Bloom</td>
<td>4/95 - 4/98</td>
<td>73,261</td>
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<tr>
<td><strong>NONFEDERAL GRANTS</strong></td>
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<tr>
<td>Barker-Welfare Foundation</td>
<td>Middle School Biology Camp/ American Museum of Natural History</td>
<td>6/95 - 6/97</td>
<td>8,650</td>
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<tr>
<td>Genentech, Inc.</td>
<td>Story of a Gene Exhibit</td>
<td>4/95 - 4/97</td>
<td>14,747</td>
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<tr>
<td>Howard Hughes Medical Institute</td>
<td>Precollege Science Education Initiative for Biomedical Research Institutions</td>
<td>7/94 - 8/99</td>
<td>82,681</td>
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<tr>
<td>Porto Conte Foundation</td>
<td>DNA Amplification and Genetic Diversity</td>
<td>2/96 - 2/97</td>
<td>20,000 *</td>
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</tbody>
</table>

The following schools each awarded a grant in 1996 for the *Genetics as a Model for Whole Learning* Program:

- China Town School District 1  $5,500
- Community School District 29  $18,400
- Great Neck Union Free School District  $8,900
- Half Hollow Hills Central School District  $5,000
- Plainedge Union Free School District  $900
- South Huntington Union Free School District  $9,600
- Syosset Central School District  $9,900

The following schools awarded a grant for *Curriculum Study* in 1996:

- of $950:
  - Commack Union Free School District
  - East Meadow Union Free School District
  - Garden City Union Free School District
  - Great Neck Public Schools
  - Half Hollow Hills Central School District
  - Harborfields Central School District
  - Herricks Union Free School District
  - Island Trees Union Free School District
  - Jericho Union Free School District
  - Lawrence Union Free School District
  - Locust Valley Central School District
  - Massapequa Union Free School District
  - North Shore Central School District
  - Oyster Bay-East Norwich Central School District
  - Plainedge Union Free School District
  - Plainview-Old Bethpage Central School District
  - Portledge School
  - Port Washington Union Free School District
  - Ramaz School
  - Roslyn Public School
  - Sachem Central School District
  - South Huntington Union Free School District
  - Syosset Central School District

- of $1,500:
  - West Hempstead

- of $2,000:
  - Hicksville Union Free School District
  - Long Beach School District

*New Grants Awarded in 1996

*Includes direct and indirect cost
Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of the State of New York. Less than half (44.1%) of our annual support is derived from Federal grants and contracts, and thus we rely heavily on support from the private sector: foundations, corporations, and individuals. Contributions from the private sector are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory has been designated a "public charity" and therefore may receive funds resulting from the termination of "private foundations."

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities: You can generally deduct the full amount of the gift on your income tax return, and, of course, you need pay no capital gains tax on the stock's appreciation.

We recommend any of the following methods:

(1) Have your broker sell the stock and remit the proceeds to Cold Spring Harbor Laboratory.

(2) Deliver the stock certificates to your broker with instructions to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.

(3) Send the unendorsed stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, New York 11724. In a separate envelope, send an executed stock power.

Pooled Income Funds: Combine gifts from a number of donors in a pool for attractive investment and tax purposes.

Appreciated real estate or personal property: Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests: Most wills probably need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

Conversion of private foundation to "public" status on termination: This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a supporting organization of Cold Spring Harbor Laboratory.

For additional information, please contact the Director of Development, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, NY 11724, or call 516-367-8840.
In 1996, the Laboratory received significant support in the form of capital and program contributions from individuals, foundations, and corporations.

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Total $6,731,224
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January 1, 1996–December 31, 1996
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Total $ 691,639
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That this was the 13th year of the Cold Spring Harbor Laboratory Corporate Sponsor Program is a testimony to both the farsightedness of its corporate members and the continuing high standards of the meetings supported by the Program. There were 35 member companies in 1996, drawn from a wide spectrum of the modern biomedical world, but having the common purpose of promoting research through supporting meetings. The Laboratory’s meetings program—in Grace Auditorium and at the Banbury Center—continues to be the premier series of meetings on molecular biology and molecular genetics. It is the firm financial support provided by the Corporate Sponsor Program that enables us to plan ahead and to hold meetings on topics that are timely and important.

The members of the Program receive special privileges in acknowledgment of their contributions: All on-site fees are waived for eight representatives of each company at our meetings. Three of these scientists may attend meetings at Banbury Center, where attendance is otherwise only by invitation of the organizers. Corporate Sponsors receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals Genes & Development, Learning & Memory, and Genome Research. Grace Auditorium is made available to Corporate Sponsor Program members for special meetings.

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Total $697,000
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An important objective of the Corporate Advisory Board of the DNA Learning Center is to provide a sustainable level of annual funding for the Learning Center's programs. As a means of reaching this objective, the Board conducts an Annual Fund and an Annual Golf Tournament with the proceeds to benefit the DNA Learning Center.

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Total $ 83,409

Total DNA Learning Center Annual Fund $ 160,704
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President's Report

As the primary support volunteer arm of the Laboratory, the Association places great emphasis upon creating new friends while maintaining solid relationships with our old friends. To this end, in 1996, members of the Association made a significant effort to reach out to persons and organizations, explaining the Laboratory’s mission and cultivating interest in the myriad activities here.

Our year began with the annual meeting on February 4. Dr. Allen Oliff, executive director for cancer research at Merck Research Laboratory, was the guest speaker. His talk, Anti-Cancer Agents Directed against Oncogene Targets, was delivered with enthusiasm and erudition. He also conveyed a sense of renewed hope for significant progress in the fight against cancer. Three directors retired; namely, Jane Greenberg, Donald Kent, M.D., and Douglas Rogers. Their unselfish service to the Laboratory is appreciated greatly. In addition, new directors were elected at this meeting, including Trudy Calabrese, Tony Kemper, and Cathy Soref.

On June 2, the annual Dorcas Cummings Lecture was sponsored by the Association. A high point of the Association's activities, our guest speaker was V. S. Ramachandran, M.D., Ph.D., professor of neuroscience at the University of California, San Diego. He discussed Illusions of Body Image in Neurology: What They Reveal About Human Nature. This stimulating and thought-provoking lecture was delivered to an enthusiastic full house. At the dinner parties held subsequent to the lecture, Dr. Ramachandran’s talk dominated the conversation. His talk demonstrated clearly that great strides are being made in neuroscience research. The dinner parties were hosted by members of the Association and other friends of the Laboratory. Visiting scientists as well as the Lab’s own scientists attended these parties—clearly an enjoyable evening. The Association wishes to express its special thanks to each of the hosts:

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In September, the Association commenced its major membership drive and annual giving effort. The results of our effort were in excess of $604,000 from 663 members. This is a good result, notwithstanding the fact that it fell short of last year’s total. The annual membership drive competed with two other major fundraising efforts which were conducted concurrently: the Emanuel Ax Gala and the Mary D. Lindsay Child Care Center Capital Campaign. The Emanuel Ax Gala was chaired by Lola Grace who, with her committee, organized a “standing room only” evening of music and dinner. The concert netted in excess of $135,000, truly a magnificent achievement. In addition, the Child Care Center Capital Campaign was commenced and, as of December 31st, had raised in cash or pledges, in excess of $620,000. This campaign is being led by the vice-president of the Association, Carol Large, who is to be commended for the great success to date. Of the funds raised for the Child Care Center, $458,000 were raised from members of the Cold Spring Harbor Laboratory Association. Thus, in the year 1996, the members of the Association have contributed more than $1 million to various projects of the Laboratory, a feat to be applauded.

In addition to our annual fund drive, a number of other activities took place in the fall. On September 24, Jennifer Jordan, a trusts and estates partner of Cadwalader, Wickersham & Taft, gave the second informative and thought-provoking estate-planning seminar. On October 13, Drs. Cynthia J. MacKay and Peter Gouras, of Columbia-Presbyterian Hospital, discussed macular degeneration, a leading cause of blindness in adults. They explained their current research in retinal cell transplants, which hopefully will lead to a new treatment for this terrible affliction. On November 17, Dr. David Grimaldi, curator and chairman of the Department of Entomology at the American Museum of Natural History, spoke to a well-attended audience on Amber: A Scientific Renaissance. His lecture held interest from many perspectives for both adults and youngsters, giving a great history of amber as well as tracing its use in jewelry.

Thanks also go to Owen and Bernadette Smith for hosting this year’s major donor cocktail party on November 24. The evening was brightened by a sampling of Owen’s cooking skills. On December 13 the Association sponsored the annual holiday tree lighting, which included caroling and holiday treats. The children had a wonderful time placing their homemade ornaments on the beautiful tree.

Throughout the year, a number of initiatives were developed by Association directors and members. These programs are on-going and involve new areas of interest; specifically, the Next Generation Initiative, a program designed to cultivate the interest of younger members of our community in the life of the Laboratory. This effort held a wonderful evening on January 18, with talks by Bruce Stillman and Tim Tully, followed by dinner parties at committee member’s homes—a very successful evening. Another program that is currently on-going is the establishment of a relationship between
schools in the area and the Laboratory. Programs have commenced that involve teachers, students, and parents from local schools to foster interest in scientific research. It is hoped that through this medium, significant contacts between scientists and students will be made.

It is with particular pleasure that I acknowledge the significant contribution given to the Laboratory during the past six years by Ed McCann and Alan Kisner, who have now stepped down as directors of the Association. In addition, our thanks also to Joyce Green, Lauri Genovese, and Bob Marcus, who served as directors of the Association and have retired. Each one of these people gave their time unselfishly and helped in many ways to further the mission of the Laboratory and its Association. For this we are very grateful. Special thanks goes to Jean Houghton, who has served enthusiastically as our staff director, and also to the other members of the Laboratory’s Development Office, who support the day-to-day efforts of the Association.

February 1997
John P. Cleary

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**CSHLA Annual Fund**

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<table>
<thead>
<tr>
<th>Category</th>
<th>Amount</th>
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<tr>
<td>Angels, Benefactors, Patrons, Associates</td>
<td>$529,903.79</td>
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<td>Friends and Basic Members</td>
<td>$72,178.71</td>
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<td>Other</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>$602,563</strong></td>
</tr>
</tbody>
</table>

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The President's Council supports our program of Cold Spring Harbor Fellows, a position created in 1986. Cold Spring Harbor Fellows are exceptionally talented, young scientists who already show the capacity for high-level, independent research while in pursuit of their doctoral degrees.

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