ONCOGENES

Structure and Activation of Mammalian *ras* Genes

K. Shimizu, M. Goldfarb, O. Fasano, E. Taparowsky, D. Birnbaum, M. Ruley, M. Wigler

DNA-transfer experiments have led to the observation that many human tumor cells contain genes capable of the tumorigenic transformation of NIH-3T3 cells. Most of these so-called "transforming genes" are members of the *ras*-gene family, a highly conserved group of genes that were first identified by their presence in the Harvey and Kirsten sarcoma viruses. We have cloned these genes from tumor cells and have been studying their nucleotide sequences, their intron/exon structures, and the predicted amino acid sequences of the proteins they encode. Additionally, we have determined the nature of the mutations that activate the transforming potential of the normal cellular genes. Further studies, utilizing techniques of in vitro mutagenesis, have provided information about the functional domains of the *ras* proteins. Finally, we have been analyzing the *ras* proteins synthesized by cells to understand the complex posttranslational processing events accompanying *ras* protein maturation.

Structure and activation of human Ki-ras *genes.* We have completed the cloning of the human Ki-*ras* gene and the sequencing of its coding regions. This large gene encompasses 45 kbp and has the potential to encode two *ras*-gene products, one of 189 amino acids homologous to the viral Ki-*ras* gene, and one of 188 amino acids. These two proteins can be encoded by mRNA that utilizes "exon choice": There are two alternate fourth coding exons. Similarly, the vestige of an "alternate" fourth coding exon is found in the 3′-untranslated sequences of the viral Ki-*ras* gene, suggesting that the rat Ki-*ras* gene also has two fourth coding exons. Except for the alternate fourth coding exon, the Ha-*ras* and the Ki-*ras* genes have a similar intron/exon structure, indicating that they have evolved from a common spliced ancestral gene. Exon-shuffling experiments show that when either alternate fourth coding exon of Ki-*ras* replaces the fourth exon of Ha-*ras*, the resulting chimeric gene is still functional. Chimeric gene constructions also show that the Ki-*ras* gene of Calu-1 lung carcinoma cells is activated by a single point mutation, resulting in the substitution of cysteine for glycine at the 12th amino acid position.

Structure and activation of the human N-ras *gene.* We have now completed the cloning and sequencing of the N-*ras* gene. Like the Ha- and Ki-*ras* genes, a functional protein of 189 amino acids is encoded by four coding exons. This gene has the same exonic structure as the Ha- and Ki-*ras* genes. We have found no evidence for an alternate fourth coding exon. Comparison of the amino acid sequences of the three human *ras* genes reveals a remarkable sequence conservation: All three are identical for the first 86 amino acids. Thereafter, until position 165, they are very similar (>80% homology). After this there is almost complete divergence, in a region we call the variable region, until again there is homology in the carboxyterminal sequence Cys-AAX, where *A* is an aliphatic amino acid and *X* is the terminal amino acid.

Comparison of the N-*ras* genes of SK-N-SH neuroblastoma cells and human placenta indicates that the former contains a mutation that activates the N-*ras* transforming potential. Chimeric gene construction, transformation assays, and nucleic acid sequence analysis reveal that this is due to a single point mutation that results in the substitution of lysine for glutamine at the 61st amino acid position. Position 12 of the transforming and wild-type N-*ras* genes is glycine.

Processing of the ras *proteins.* Analysis of the predicted amino acid structure of the *ras*-gene products suggests that the carboxyterminal variable domain is the candidate determinant for physiologic specificity of the *ras* proteins. However, it was shown several years ago that the *ras* proteins undergo processing in the carboxyterminal two thirds of the molecule. This processing is visualized by a marked increase in the electrophoretic mobility of mature *ras* protein, suggestive of protein cleavage at the carboxyl terminus. This putative cleavage event would remove the variable region, making it unlikely that it could have more than a transient function. We therefore prepared two-dimensional tryptic cleavage maps of the processed and unprocessed Ha-*ras* proteins to resolve this

question. These studies indicate that the processing event is not the result of proteolytic cleavage and that the variable region is present on mature processed *ras* proteins. Work is continuing to determine the nature and location of the processing event. Preliminary data indicate that the same processing event or a similar one occurs when the mammalian Ha-*ras* proteins are expressed in yeast cells (see below).

Activating mutations of the Ha-ras *gene*. All of the activating mutations of the *ras* genes that occur in vivo appear to be point mutations resulting in amino acid substitutions at the 12th or 61st codons. There are at least nine examples of these reported by our laboratory and others. However, they are not necessarily the only mutations that can activate the transforming potential of the *ras* genes. To test this, we randomly mutagenized a wild-type Ha-*ras* gene by the bisulfite method, introducing C→T transitional mutations. By screening the resulting mutants in NIH-3T3 transformation assays and sequencing the transforming mutants, we found that point mutations leading to amino acid substitutions at positions 13, 59, and 63 can also activate the transforming potential of the *ras* genes. Not all mutants are equally effective in transformation. Mutants encoding aspartic acid for glycine at position 13 are perhaps one-fifth as potent as those encoding valine for glycine at position 12. Mutants encoding serine for glycine at position 13 are perhaps 1/100 as potent. Mutants encoding threonine for alanine at position 59 are particularly potent. This is puzzling, since both Ha-*ras* and Ki-*ras* viral genes have at least two transforming mutations: one encoding substitutions at position 12 and one encoding threonine at position 59. Furthermore, it is no longer clear why only position-12 and position-61 mutants are found in vivo.

The clustering of activating mutations has two other important implications. First, these results suggest that activating mutations disrupt some critical *ras* protein function by disrupting protein structure. We have argued that these mutations occur about the guanine-nucleotide-binding site of the *ras* protein. From this, we have further argued that activating mutations alter the conformational changes induced by guanine nucleotide binding. Second, it may now be easier to screen tumor DNAs to determine whether they contain activating mutations using methods of hybridization.

Expression of the Human Ha-*ras* Gene in *Escherichia coli* and Yeast

S. Powers, M. Goldfarb, O. Fasano, M. Wigler

Biochemical study of the *ras* proteins requires a source from which a functional *ras* protein can be purified in abundance. In collaboration with M. Rosenberg, R. Sweet, and M. Gross at Smith, Kline and French Laboratories and J. Feramisco and T. Kamata at Cold Spring Harbor, we have utilized an efficient λ promoter cloned into a pBR322 derivative to express clones of normal and mutant Ha-*ras* cDNAs. *Escherichia coli* harboring these vectors express Ha-*ras* in great abundance. This protein comigrates with the unprocessed Ha-*ras* protein made in animal cells and also efficiently binds guanine nucleotides. Ha-*ras* synthesized and purified from *E. coli* can now be used to study the effects of mutation on *ras* protein structure, to raise monoclonal antibodies, and to study *ras* function by microinjection. Since *ras* made in *E. coli* is unprocessed, we have examined the processing of Ha-*ras* expressed in yeast. In collaboration with J. Broach (SUNY, Stony Brook), we constructed a galactose-inducible expressor of the Ha-*ras* cDNA. *Saccharomyces cerevisiae* containing this vector can be induced to express an Ha-*ras* protein that undergoes a time-dependent processing event similar to, but not identical with, the processing events in animal cells. Thus, yeast expression systems may be used to purify large amounts of the processed Ha-*ras* protein for biochemical studies and also to study the signals for protein processing.

Yeast *ras* Genes

S. Powers, T. Kataoka, O. Fasano, M. Goldfarb, M. Wigler

Since the *ras* genes are so well conserved in evolution, being present even in *Drosophila*, we looked for their existence in *Saccharomyces cerevisiae*, a simple eukaryotic organism that can be subjected to powerful genetic analysis. We have found two homologous genes in yeast by Southern blot analysis, have cloned both from plasmid libraries, and have completed DNA sequence analysis of the coding regions. The predicted amino acid sequence was compared with that of the human *ras* genes, with striking results. Both yeast genes show almost 90% homology with the first 80-amino-acid positions of the mammalian *ras* and 50% homology with the next 80. Although the yeast *ras* genes encode much larger proteins (315 amino acids for yeast *RAS1* and 322 amino acids for *RAS2*), they both terminate with the sequence Cys-AAX, common to mammalian *ras*. Thus, there are clear domains of conservation that we think reflect functional domains of the encoded proteins. Although the two yeast genes are far closer to each other than to the mammalian *ras*, they diverge radically from each other at precisely the point where the mammalian variable region begins. We have hypothesized that the first 80 amino acids comprise the effector domain of the *ras* proteins and that the variable region forms a domain that functions as a physiologic receptor.

The availability of powerful genetic tools in yeast has enabled us to begin a series of experiments probing the function of yeast *ras* genes. This work was carried out with the collaboration of J. Strathern (Yeast Section) and J. Broach (SUNY, Stony Brook). We have found that yeast cells with a deletion of either yeast *RAS1* or *RAS2* are viable; deletion of both genes is lethal. By site-directed mutagenesis, we have introduced the activating valine for glycine substitution in *RAS2* at the position homologous to the mammalian *ras* position 12. Cells containing this mutant have a drastically altered phenotype: They grow poorly on certain media, are flocculant, and do not appear to arrest properly in G_1. Diploids with this mutation do not sporulate. This phenotype is dominant.

These experiments indicate that it will be possible to do detailed genetic studies of the yeast *ras* genes and that they may have cellular functions that are homologous to their mammalian counterparts. In particular, failure to arrest properly in G_1 is a hallmark of transformed animal cells.

Tumorigenicity Assay for Transforming Genes

O. Fasano, D. Birnbaum, C. Birchmeier, L. Edlund, M. Wigler

All of the transforming genes we have detected in tumor-cell DNA using the NIH-3T3 focus assay have been members of the *ras*-gene family. All of these genes have had mutations that introduced structural alterations into the *ras* proteins. We therefore suspected that the NIH-3T3 focus assay was biased in its sensitivity to structurally mutated *ras* genes. In collaboration with J. Fogh (Sloan-Kettering Institute, Rye, New York), we have developed and applied an alternative assay for transforming genes present in NIH-3T3 cells after DNA transfer: tumorigenicity in nude mice. NIH-3T3 cells are cotransfected with a selectable marker in the presence of tumor DNA and then assayed for tumorigenicity. We found that many more DNAs are positive in this assay than in the focus assay. We have identified and cloned three transforming genes in the DNA of the human breast carcinoma cell line, MCF-7, which did not induce transformed foci of NIH-3T3 cells in our hands. One of these genes is N-*ras*, and we find N-*ras* to be amplified in MCF-7 cells. The other two genes have no apparent relationship to previously known viral or cellular oncogenes. We have not yet established whether these human genes are genetically altered in MCF-7. These results raise anew the question of the number of potential oncogenes in our genome and indicate that some (perhaps most) tumor cells may contain a multiplicity of weakly transforming genes.

Cooperation among Oncogenes

D. Birnbaum, L. Edlund, M. Wigler

Recent observations by E. Ruley at Cold Spring Harbor and H. Land and others at the Massachusetts Institute of Technology indicate that certain oncogenes can cooperate in the morphologic and tumorigenic transformation of primary embryo cells. In particular, a mutant *ras* gene appears to be insufficient to induce full transformation but requires in addition the presence either of high-level expression of *myc* or adenoviral E1A or of another suitable viral gene. We have confirmed these observations concerning the cooperation of *myc* and *ras* and, in particular, have shown that high-level expression from a normal human *myc* gene is sufficient to complement a mutant *ras* gene in the tumorigenic transformation of primary rat embryo fibroblasts. As in the work of Land, we observed that mutant *ras* alone is sufficient to induce morphological changes in primary embryo cells, but that such cells have only a very limited life span. Indeed, we observed that primary embryo fibroblasts containing mutated *ras* genes have a greatly reduced life span compared with normal embryo fibroblasts, and we interpret this to mean that, in the absence of high-level expression of *myc* function, mutant *ras* genes are actually toxic to these primary cells. We have also tested the cooperation between *myc* and various other viral oncogenes using the primary rat embryo fibroblast transformation system. So far, we have observed cooperative interactions between *sarc* and *myc* and, surprisingly, none between *myc* and a variety of other tyrosine kinases or between *myc* and *mos*. We also observed a cooperative interaction between *myc* and *fos*, an oncogene encoding a nuclear protein.

Biochemistry of *ras* and *ras*-related Proteins

F. Tamanoi, M. Walsh, M. Rao

Mutations in *ras* genes are known that are responsible for the alteration of a normal protein to a form active in transformation of NIH-3T3 cells. An intriguing question is, what biochemical activities are affected by these mutations? To obtain an answer, it is necessary to carry out a detailed enzymological study, as well as a conformational and crystallographic study, of the protein. One of the most promising ways to obtain pure *ras* protein for such a study is to express the protein in *Escherichia coli*. Therefore, during the past year we have concentrated on expressing *ras* protein in *E. coli* using various expression vectors.

First, we have attempted to express mammalian *ras* protein using the *lac* promoter. Using a unique *Mst*I site five nucleotides upstream of ATG, we inserted the *ras* gene downstream from the *lac* pro-

moter in M13mp10. The recombinant phage directed the production of a 23K fusion protein that was immunoprecipitable with a monoclonal antibody against the mammalian *ras* protein. The level of expression could be increased to 5–10% of the total protein by transferring into a pUC vector. The protein made in *E. coli* is active in binding GDP. Recently, a *ras* expression system that utilizes the λ P_L promoter has been constructed by M. Wigler (this section) in collaboration with M. Rosenberg (Smith, Kline and French Laboratories) and J. Feramisco. This system has the advantage that an intact protein is expressed. Characterization of this intact protein is discussed by J. Feramisco et al. (this section).

Recently, two yeast genes (*RAS1* and *RAS2*) that exhibit remarkable homology with the mammalian *ras* gene have been isolated (see M. Wigler, this section). To investigate the biochemical activity of these gene products, we have started to express the gene products in *E. coli*. The *RAS2* gene contains a unique *Hpa*I site just upstream of ATG that could be used to insert the gene into expression vector pUC. The recombinant plasmid produced two proteins, 46K and 35K, that could be immunoprecipitated by the monoclonal antibody against the mammalian *ras* protein. The relationship between the two proteins is currently being investigated. With the *RAS1* protein, no restriction sites suitable for cloning were found around ATG. Therefore, we have created a *Sph*I restriction site (GCATGC) at the ATG by a single base change. This was possible by cloning the *RAS1* gene into bacteriophage M13 and by using a synthetic oligonucleotide. Using this *Sph*I site, we are inserting the *RAS1* gene into vectors that contain P_R or P_L promoters of bacteriophage λ. Our immediate goal is to see whether yeast *ras* proteins exhibit any GTP-binding activity and, if they do, to compare the activities between the two yeast proteins, as well as to the mammalian protein.

Yeast provides an attractive system to carry out biochemical analyses of *ras* proteins. Powerful yeast genetics could lead to the identification of proteins that interact with the *ras* proteins (see M. Wigler, this section). These interacting proteins could be expressed in *E. coli* to carry out studies on the protein-protein interaction. Mutation of *ras* protein to a form active in transformation might involve alteration of such a protein-protein interaction.

Microinjection of Cloned Genes to Study the Mechanisms of Transformation

B.R. Franza, Jr., H.E. Ruley, J.I. Garrels

We are studying the effects of cloned oncogenes introduced into defined populations of cells utilizing the techniques of microinjection, photomicroscopy, and computer analyzable two-dimensional gel electrophoresis. Initial studies have focused on the cloned Ha-*ras*-1 (pT24) transforming gene from the human bladder carcinoma line T24 (Fasano et al., *J. Mol. Appl. Genet. 2:* 173 [1983]). We found that this gene will not transform REF52 cells (a rat-embryo-derived line; McClure et al., *Cold Spring Harbor Conf. Cell Proliferation 9:* 345 [1982]) unless other genes such as the Ad5 E1A region are cointroduced into the cell either by calcium phosphate transfection or by microinjection. Despite the failure of the T24 gene product to transform, it is abundantly expressed in microinjected REF52 cells. At 72 hours postinjection, the protein product is easily detected on two-dimensional gels (Fig. 1); however, no major alteration of cell morphology is apparent.

Another cloned oncogene is capable of rapid and dramatic morphological transformation of REF52 cells. Less than 24 hours after injection of the cloned *c-sis* gene—cloned from HUT-102 cells, a T-cell lymphoblastoid cell line that produces a human T-cell leukemia virus (Poiesz et al., *Proc. Natl. Acad. Sci. 77:* 7415 [1980]), and kindly provided, as part of an ongoing collaboration, by S. Josephs, F. Wong-Staal, and R. Gallo (National Cancer Institute)—many cells have changed their morphology and their growth rate, leading to formation of a focus within 72 hours of the time the gene was injected (Fig. 2). We are intrigued by the response of REF52 cells to the *c-sis*-gene product given the fact that considerable homology exists between the protein product of *v-sis* (the simian sarcoma virus *onc* gene) and a platelet-derived growth factor (PDGF) (Deuel et al., *Science 221:* 1348 [1983]; Doolittle et al., *Science 221:* 275 [1983]); and yet PDGF will not support growth of normal REF52 cells.

We intend to continue such studies as these and utilize the resolution capacity of the two-dimensional gel and QUEST computer analysis (Garrels et al., in *Two-dimensional Gel Electrophoresis of Proteins*, Academic Press, New York [1984]) of the protein patterns to begin defining the early events that occur in changes of protein synthesis and turnover as cells respond to the products of genes known either to transform or to assist in the transformation process.

Production of Monoclonal Antibodies to the *ras* Proteins

T. Kamata, C. Fraser, G. Binns, K. Ramirez, J.R. Feramisco

In a collaborative effort utilizing the human *c*-Ha-*ras* genes cloned in M. Wigler's laboratory, an efficient protein expression system of M. Rosenberg and colleagues (at Smith, Kline and Beckman Laboratories), and the combined use of ours and Smith, Kline and Beckman's protein-purification techniques and facilities, we have obtained homoge-

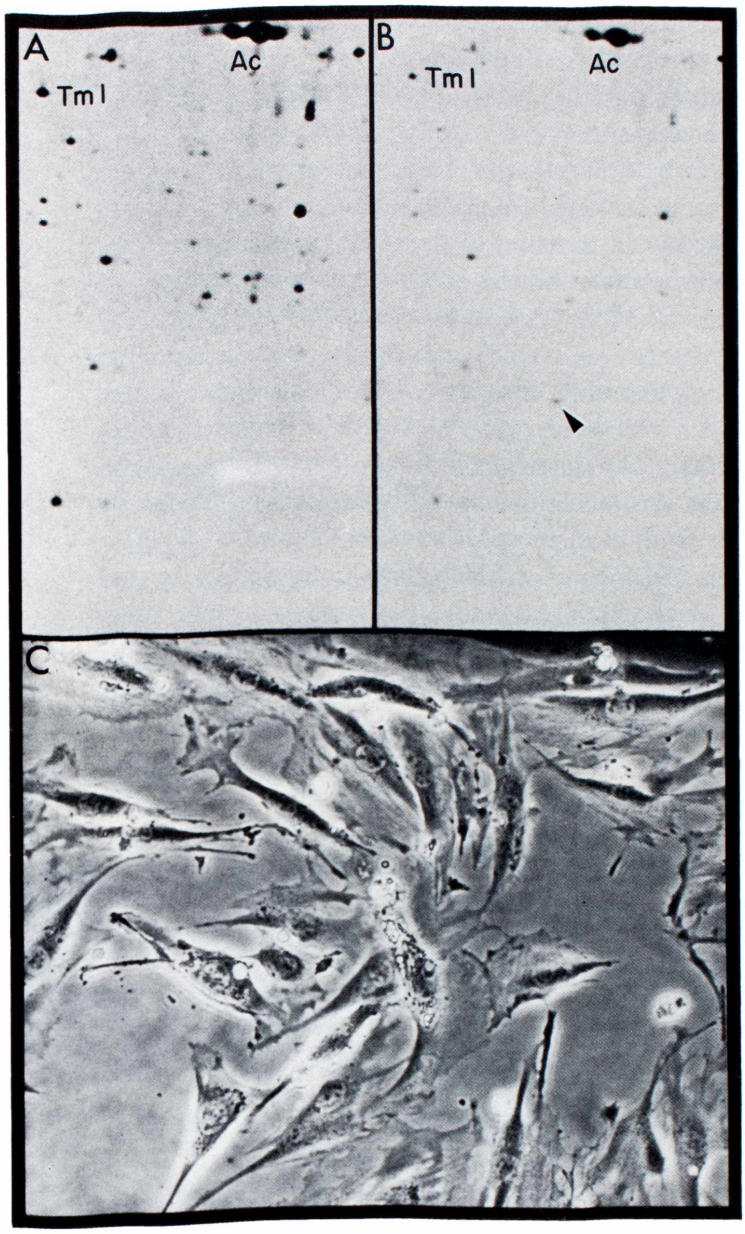

Figure 1
Expression of pT24 gene in REF52 cells. Subconfluent REF52 cells were labeled for 2 hr with [^{35}S]methionine, 72 hr after introduction of the pT24 gene by microinjection. (*A*) Proteins resolved from control, noninjected cells; (*B*) proteins resolved from cells injected with pT24; (*C*) phase photomicrograph of pT24-injected cells just prior to preparation for two-dimensional gel analysis. (Ac) Actin; (Tm1) tropomyosin 1. Arrow indicates the synthesis of the T24 gene product by the injected cells.

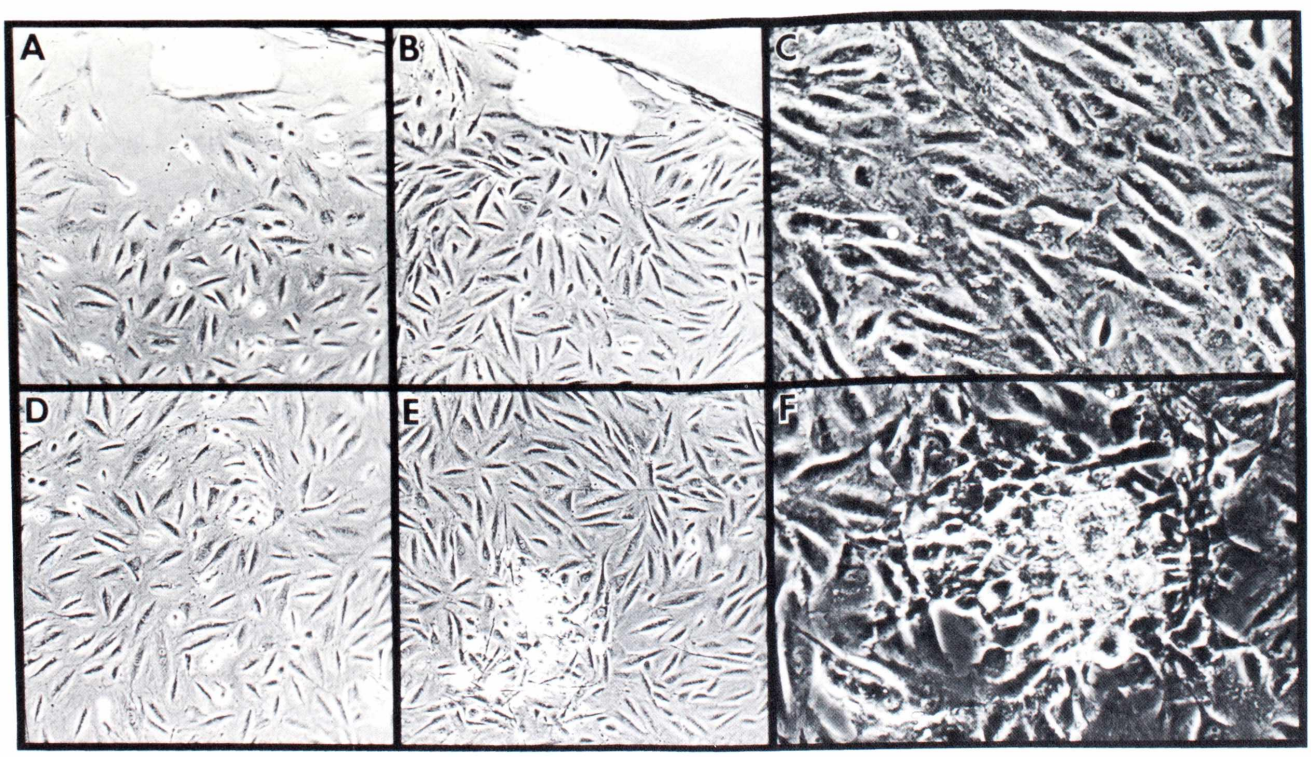

Figure 2
Morphological transformation of REF52 by the *c-sis* oncogene. Confluent (stringent condition for inducing transformation) REF52 cells were injected with the plasmid containing the *sis* gene (pSM1) and photographed at 24 hr and 72 hr after microinjection. (*A–C*) Noninjected controls for each time point. Cells microinjected with the vector minus the *c-sis*-gene insert are indistinguishable from noninjected cells at each time point. (*D–F*) Phase photomicrographs of the pSM1-injected cells at 0, 24, and 72 hr, respectively. Note that *C* and *F* are photographed at higher magnification.

neous, biochemically active preparations of several protein forms of the human *ras* proto-oncogenes and oncogenes, including the "activated" *c*-Ha-*ras* product, the "wild-type" *c*-Ha-*ras* product, and a carboxyterminal truncate of the activated *c*-Ha-*ras* protein lacking 19 amino acids.

Using in the first case a readily obtainable aminoterminal truncate (−20 amino acids) as an antigen, we have prepared several hundred mouse hybridoma cell lines secreting antibodies that recognize the antigen. Of these, approximately seven have been cloned through soft agar to homogeneity. At least five of these cloned lines produce IgGs that are capable of immunoprecipitating the *ras* protein in the presence or absence of denaturants (SDS) and are being characterized as to their relative specificities toward the other protein members of the *ras* family and to their effects on the guanine-nucleotide-binding reaction characteristic of the *ras* proteins.

In addition, we produced two 18-residue synthetic peptides corresponding to the two aminoterminal sequences that differ by one residue between the activated and proto-oncogenic forms of the human Ha-*ras* proteins. Smith, Kline and Beckman have provided us with a 22-residue peptide that corresponds to the carboxyl terminus of the human *c*-Ha-*ras* proteins. All of these peptides will be used as antigens and, with luck, might provide us with some very useful antibodies.

Microinjection of the Purified Human Forms of the Activated and Normal Cellular *ras* Proteins into Living Cells

J.R. Feramisco, T. Kamata, G. Binns

As mentioned above, we have made homogeneous preparations of the full-length forms of the human

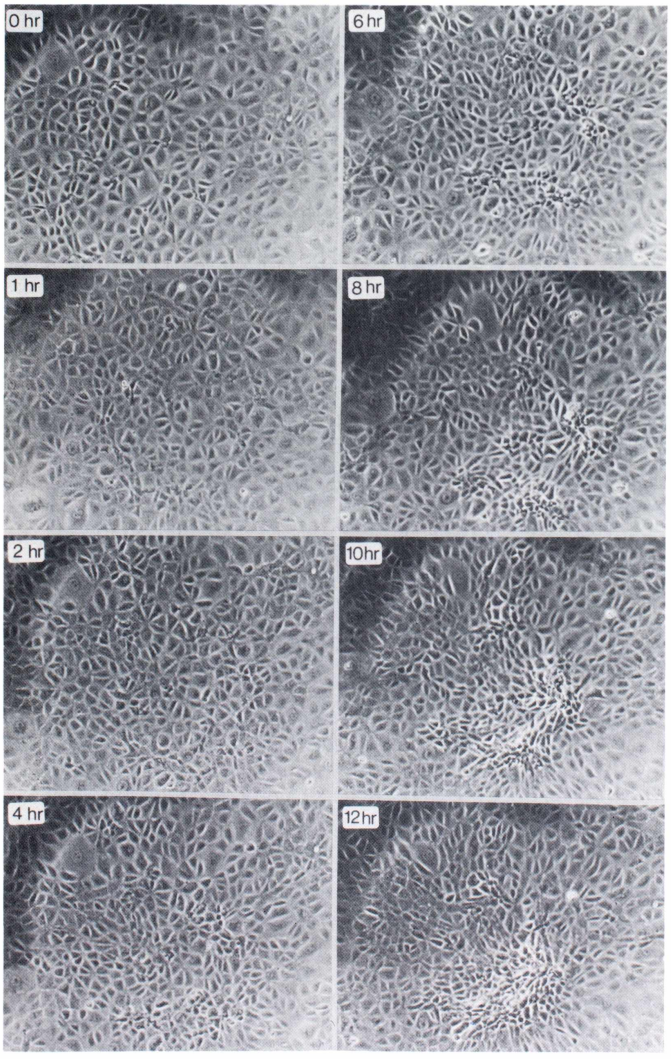

Figure 3
Approximately 30 NRK cells in the center of the field were each injected with approximately 10^6 molecules of the human activated Ha-*ras* protein (full length) purified after expression in *E. coli*. Photographs of the area were taken right after injection (0 hr) and as indicated in the frames.

Ha-*ras* proteins (both the wild-type or proto-oncogenic form and the activated or oncogenic form), as well as an activated protein lacking the carboxy-terminal 20 or 50 amino acids. All of these proteins seem to be "native" and are capable of binding guanine nucleotides (to at least the 30% level) by several different types of assays. M. Gross, S. Yokoyama, R. Sweet, M. Rosenberg, and colleagues (Smith, Kline, and Beckman Laboratories) are in the process of quantitating the binding properties of these proteins more exactly.

We have begun studies utilizing these purified proteins and microinjection into living, normal cells in order to gain more insight into the function of the human *ras* oncogenes. The initial results of these experiments show some very promising features. As shown in Figure 3, within several hours after injection of the oncogenic form of the human Ha-*ras* protein into confluent NRK cells, dramatic morphological changes in the cells can be detected. Microinjection of high levels of the normal, proto-oncogenic form of the *ras* protein has a slight effect on the cells, but nothing as obvious as the effect of the activated protein. The carboxyterminal truncate of the activated protein appears to have no effect on the cells. Video time-lapse recording of the cells after microinjection of the activated *ras* protein indicated numerous cell divisions and incredibly active cell movements such as blebbing and ruffling. Other experiments indicate that the effect of the *ras* protein requires entry into the cells, is temporary, does not require the presence of serum, and is inhibited by cycloheximide or actinomycin D. We are in the process of trying to understand this effect in molecular detail.

Is the *ras* Oncogene Protein a Component of the Epidermal Growth Factor Receptor System?

T. Kamata, G. Binns, J.R. Feramisco

A number of human cellular oncogenes have been identified in part by transfection of DNA from tumor-cell lines and tumor tissues into NIH-3T3 cells. Several of these oncogenes have been classified as members of the *ras*-gene family because of their relatedness to the viral oncogenes of Harvey (*v*-Ha-*ras*) or Kirsten (*v*-Ki-*ras*) murine sarcoma virus (Ha-MSV or Ki-MSV). The human genes have been put into at least three classes, *c*-Ha-*ras*, *c*-Ki-*ras*, or *c*-N-*ras*, depending on the degree of homology with *v*-Ha-*ras*, *v*-Ki-*ras* oncogenes, or the human cell line of initial description (i.e., neuroblastoma), respectively. All of the known *c-ras* oncogenes have normal or proto-oncogene forms that have only a single amino acid change from the corresponding activated oncogene forms. In general, the proteins encoded by the *ras*-gene family are approximately 21,000 daltons and are associated with the inner-surface plasma membrane. The only known biochemical property common to all forms of the *ras* proteins is the ability to bind guanine nucleotides. In the particular case of the *v*-Ha-*ras* protein, apparent autophosphorylation on threonine occurs (both in vivo and in vitro). The guanine-nucleotide-binding property is most likely an important property of the *ras* proteins, since in a temperature-sensitive viral mutant of the *v*-Ki-*ras* gene, the virus is temperature sensitive for transformation and the *v*-Ki-*ras* protein is temperature sensitive for the guanine-nucleotide-binding activity. Of the many alterations in the cellular phenotype associated with transformation by the *ras* oncogenes, the one relevant to the present work is the apparent loss of epidermal growth factor (EGF)-binding sites on the surfaces of the transformed cells. This has been postulated to occur as a result of either down-regulation of the receptors or occupancy of the receptors by the alpha-type transforming growth factor (TGFα), which is produced by the transformed cells. Because of the similar cellular location of the *ras* oncogene proteins and the growth factor receptors, and because several other hormone systems utilize guanine-nucleotide-binding proteins as regulatory elements (such as the *G* protein of the adenylate cyclase system), we investigated the possible relationship of *ras* oncogene proteins to the EGF receptors. We have found that the normally low EGF-binding activity of *ras*-transformed cell membranes is apparently stimulated by the addition of guanine nucleotides. By immunoadsorption of the *ras* proteins from the membranes, we found that this effect appears dependent on the presence of the *ras* protein. In addition, we have found that the phosphorylation of the *v*-Ha-*ras* protein and the guanine-nucleotide-binding activity of the *v*- or *c*-Ha-*ras* proteins in membranes isolated from transformed cells are stimulated by the addition of EGF and $MgGTP^{--}$. Taken together, these results suggest a role for the *ras* oncogene protein in the EGF receptor system, perhaps acting in a manner similar to the *G* protein.