GENETICS OF CELL PROLIFERATION

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Our laboratory is concerned with the genetic and functional analysis of growth control in eukaryotic cells. We have stressed studies on the function of the *ras* oncogenes, a family of genes found to be activated in perhaps 10–20% of human cancers and which have been highly conserved in evolution. Homologs of the mammalian *ras* exist in the yeast *Saccharomyces cerevisiae* (*RAS1* and *RAS2*), and we have been studying the function of these genes in this species. We have shown that human *ras* proteins can complement the loss of *RAS1* and *RAS2* proteins in yeast and hence are functionally homologous. Both human and yeast *ras* proteins can stimulate the magnesium and guanine nucleotide-dependent adenylate cyclase activity present in yeast membranes, and hence we also have been studying the adenylate cyclase pathway of yeast. Our studies have led to the important conclusion that although *ras* is an essential controlling element of adenylate cyclase in yeast, it has other essential functions in that organism. Moreover, there may be multiple pathways controlling the growth of yeast that are independently regulated but can have overlapping functions. In this respect, growth control in

yeast may be fundamentally as complex as growth control in mammalian cells and may prove to be a useful model system for studying the latter.

In addition to our studies of yeast, we are continuing studies on oncogenes in mammals. We have developed a useful in vivo assay system for the human *ras* proteins: induction of maturation in microinjected frog oocytes. From these studies, we conclude that *ras* proteins do not stimulate the adenylate cyclase of frog oocytes but have powerful biological effects through another pathway. We are studying two other human oncogenes: the human *ros1* gene, which may be a growth-factor receptor, and the human *mas1* gene, which is a new oncogene we discovered that encodes a protein of novel structure.

Studies in *Saccharomyces cerevisiae*

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We have previously demonstrated the existence of two *ras* genes in the yeast *Saccharomyces cerevisiae, RAS1* and *RAS2*, which encode proteins that are structurally homologous to the mammalian *ras* proteins (Powers et al., *Cell 36:* 607 [1984]). The studies described below demonstrated that *ras* genes are essential for *S. cerevisiae,* that they are essential controlling elements of adenylate cyclase in *S. cerevisiae,* that the mammalian *ras* can perform this biochemical function, and that yeast *RAS* genes have at least one other essential function in *S. cerevisiae.* The latter result derives from two experimental sources: studies of suppressors of *ras* function and careful genetic analysis of the adenylate cyclase pathway in yeast. We have isolated and characterized several of the genes of the *ras*/adenylate cyclase pathway, some of still unknown function.

EARLY STUDIES OF YEAST *RAS* GENES

We and other investigators have previously shown that at least one functional *ras* gene is essential for the germination of haploid yeast spores (Kataoka et al., *Cell 37:* 437 [1984]; Tatchell et al., *Nature 309:* 523 [1984]). This was demonstrated by constructing doubly heterozygous diploid yeast cells containing wild-type *RAS1* and *RAS2* alleles and *RAS1* and *RAS2* alleles each disrupted by a different auxotrophic marker. These diploid cells were then sporulated, and the resulting tetrads were analyzed. Only spores containing at least one functional *ras* gene could germinate. We then introduced into these doubly heterozygous diploid cells a *RAS2* gene under the transcriptional control of the galactose-inducible *GAL10* promoter (Kataoka et al., *Cell 40:* 19 [1985b]), linked to a third auxotrophic marker. These diploid cells were then sporulated, and tetrads germinated on either glucose-containing medium (YPD) or galactose-containing medium (YPGal). The results confirm that at least one functional *ras* gene is required for germination. *ras1*$^-$ *ras2*$^-$ *GAL10-RAS2* spores germinate only in the presence of galactose, the inducer of the *GAL10* promoter. Cells with the genotype *ras1*$^-$ *ras2*$^-$ *GAL10-RAS2* were grown in YPGal and then shifted to YPD. Within several cell generations, cell growth ceased and cells lost viability. These experiments indicated that the *ras* genes are needed not only for spore germination, but also for the continued growth and viability of yeast cells (Kataoka et al., *Cell 40:* 19 [1985b]).

Using the approach described above, we have been able to test if expression of the normal human Ha-*ras* protein is sufficient for viability in yeast cells lacking their own endogenous *ras* genes. To this end, we constructed a *GAL10*-Ha-*ras* transcription unit that utilized a full-length cDNA clone of the human Ha-*ras* mRNA under the control of the galactose-inducible *GAL10* promoter. This unit, closely linked to a *LEU2* marker, was inserted into diploid yeast cells that were doubly heterozygous for their endogenous *ras* genes. Cells were induced to sporulate, and tetrads were examined after germination on YPD or YPGal plates. Approximately 40% of spores with the genotype *ras1*$^-$ *ras2*$^-$ *GAL10*-Ha-*ras* were capable of germination when plated on YPGal, from which we conclude that the human Ha-*ras* protein can supply essential *ras* function to yeast.

Certain missense mutations drastically alter the biological activity of mammalian *ras* genes. In particular, the human Ha-*ras*^{val12} gene, which encodes valine instead of glycine at the 12th codon of the Ha-*ras* gene, can induce the tumorigenic transformation of NIH-3T3 cells. To test the consequences of a similar mutation of *RAS2* on the properties of

yeast cells, we constructed the *RAS2*^{val19} gene using site-directed mutagenesis (Kataoka et al., *Cell 37:* 437 [1984]). *RAS2*^{val19} thus encodes valine instead of glycine at the 19th codon, which corresponds to the 12th codon of the mammalian Ha-*ras* gene. We then examined the effects of introducing this gene into yeast cells. Our first observation was that diploid cells containing *RAS2*^{val19} could not be induced to sporulate by incubation under conditions of nutritional deprivation (Kataoka et al., *Cell 37:* 437 [1984]). Next, we observed that haploid cells carrying *RAS2*^{val19} lost viability if starved for nitrogen, sulfur, or phosphorus and failed to arrest in G_1 under those conditions (Toda et al., *Cell 40:* 27 [1985]). Wild-type cells normally arrest in G_1 and retain viability for long periods if nutritionally deprived. In addition to these phenotypes, we also noted that cells carrying *RAS2*^{val19} failed to accumulate carbohydrate stores as cells entered the stationary growth phase. Thus, in general, *RAS2*^{val19} cells appeared to show a defective response to nutritional stress (Toda et al., *Cell 40:* 27 [1985]).

The cluster of cellular properties just described closely resembles the phenotype of cells carrying the *bcy1* mutation (Matsumoto et al., *Proc. Natl. Acad. Sci. 79:* 2355 [1982], *Cell 32:* 417 [1983], *Exp. Cell Res. 146:* 151 [1983]; Uno et al., *J. Biol. Chem. 257:* 14110 [1982]). The *bcy1* mutation was first isolated by Matsumoto and co-workers as a mutation that suppresses the growth defect that otherwise results from the disruption of adenylate cyclase (Matsumoto et al., *Proc. Natl. Acad. Sci. 79:* 2355 [1982]). Cells carrying *bcy1* appear to lack the regulatory subunit of the cAMP-dependent protein kinase and hence have lost the requirement for cAMP (Uno et al., *J. Biol. Chem. 257:* 14110 [1982]). These observations suggested that the *ras* genes might be participating in the cAMP pathway. In support of this idea, we found that *bcy1* suppressed the lethality that otherwise results from disruption of both *ras* genes.

The adenylate cyclase activity of the yeast *S. cerevisiae* is stimulated by guanine nucleotides in the presence of magnesium (Casperson et al., *J. Biol. Chem. 258:* 791 [1983]). In this respect, yeast adenylate cyclase resembles the adenylate cyclase of mammalian cells, which can be stimulated by a guanine-nucleotide-binding complex called G_s (Gilman, *Cell 36:* 577 [1984]). Since the yeast *ras* proteins also bind guanine nucleotides (Tamanoi et al., *Proc. Natl. Acad. Sci. 81:* 6924 [1984]), we reasoned that they might also modulate adenylate cyclase. This was tested directly by the assay of membranes from wild-type yeast cells and *ras1*$^-$ *ras2*$^-$ cells. Membranes from wild-type or *ras1*$^-$ *ras2*$^-$ strains contained appreciable adenylate cyclase activity when assayed in the presence of manganese ion, but *ras1*$^-$ *ras2*$^-$ membranes displayed negligible levels of activity when assayed in the presence of magnesium and a nonhydrolyzable guanine nucleotide analog (Toda et al., *Cell 40:* 27 [1985]). These results were confirmed in a striking manner by membrane-mixing experiments. We prepared membranes from *RAS1 RAS2* yeast carrying the *cyr1-1* mutation and membranes from *ras1*$^-$ *ras2*$^-$ yeast. The *cyr1-1* mutation disrupts the catalytic subunit of adenylate cyclase. Membranes from these two sources were assayed separately and together after membrane mixing and fusion. The data indicate that membrane mixing and fusion regenerate a guanine-nucleotide-stimulated adenylate cyclase activity (Toda et al., *Cell 40:* 27 [1985]).

These studies suggested that it would be possible to develop an in vitro assay for *ras* protein function. The addition of purified yeast *RAS2* protein to membranes from *bcy1 ras1*$^-$ *ras2*$^-$ cells indeed induces adenylate cyclase activity to about 50-fold above background level (Broek et al., *Cell 41:* 763 [1985]). Adenylate cyclase activity in the *bcy1 ras1*$^-$ *ras2*$^-$ membranes can also be increased dramatically by addition of yeast *RAS1* protein. Restoration of adenylate cyclase activity by *ras* proteins is dependent on the presence of guanine nucleotide, with the nonhydrolyzable GTP analog, GppNp, yielding twice the activity of that observed in the presence of GDP. Incubation of the *RAS2* protein with GTP prior to mixing with the *bcy1 ras1*$^-$ *ras2*$^-$ membranes results in adenylate cyclase activity comparable to that restored with *RAS2* bound to GDP. In contrast, preincubation of *RAS2*^{val19} with GTP results in activation of adenylate cyclase to levels identical with those induced by *RAS2* proteins bound to GppNp. These results confirm our genetic studies and indicate that there are proteins in membranes which can distinguish between *ras* proteins complexed with GTP and *ras* proteins complexed with GDP; furthermore, they provide for the first time an in vitro bioassay for the effector function of *ras* (Broek et al., *Cell 41:* 763 [1985]).

Using this approach, we tested whether the human *ras* protein could stimulate yeast adenylate cyclase. For this purpose, we used a bacterial expression system as our source of human Ha-*ras* protein (Gross et al., *Mol. Cell. Biol. 5:* 1015 [1985]). Adeny-

late cyclase activity in *bcy1 ras1*$^-$ *ras2*$^-$ membranes was dramatically increased by the addition of purified human Ha-*ras* protein. These results indicate that the effector domain of yeast and mammalian *ras* proteins has been conserved in evolution (Broek et al., *Cell 41:* 763 [1985]). In particular, these results urged us to test whether *ras* proteins may be involved in regulating adenylate cyclase in vertebrates. However, these experiments, conducted with *ras*-injected frog oocytes, indicated that *ras* was not acting upon adenylate cyclase but upon some other effector system (Birchmeier et al., *Cell 43:* 615 [1985]; and see below).

The results of our studies in yeast and frog oocytes have left us with an apparent paradox: *ras* stimulates adenylate cyclase in one but not in the other. Several models are consistent with these results. In the first model, *ras* proteins interact directly with a domain of yeast adenylate cyclase. In the course of evolution, the interaction with this domain has been conserved, but the function of adenylate cyclase has changed. In a second model, *ras* interacts directly with another component (X) that directly or indirectly interacts with adenylate cyclase in yeast. In the course of evolution, the interaction of *ras* with X has been conserved, but X interacts with a different effector system in vertebrates. In a third model, *ras* interacts directly or indirectly through more than one effector system, and in evolution, one or more of these interactions have been conserved and/or are operant in different cell types. To decide among these possibilities, we have initiated more detailed studies in yeast. The first major approach has been a detailed genetic analysis of the adenylate cyclase pathway (described in the next section). The second major approach has been an examination of *ras* function by "suppressor" analysis (described in the section following the next).

THE YEAST ADENYLATE CYCLASE PATHWAY

Adenylate Cyclase (CYR1). Mutants in the adenylate cyclase locus, *CYR1*, were obtained from K. Matsumoto. The wild-type gene for *CYR1* was cloned by complementation screening and then sequenced. It contains an open reading frame of greater than 6000 bp, which can encode a protein of 2026 amino acids (Kataoka et al., *Cell 43:* 493 [1985a]). Northern analysis indicates that the entire open reading frame is used. The structure of this large protein is interesting. Centrally located is a 23-amino-acid unit repeated 25 times. The consensus sequence of this repeat is leucine-rich, and, amazingly, a protein with a similar consensus sequence and periodic structure is found in human serum. Deletion analysis indicates that the catalytic activity is located in the carboxyterminal portion of the molecule, and the aminoterminal portion contains the *ras* responsive domain.

High-level expression of the catalytic portion of adenylate cyclase in yeast is sufficient to suppress the lethality that results from loss of *ras* function. Thus, if *ras* has other essential functions in yeast besides stimulating adenylating cyclase, these functions can be compensated by overexpression of the cAMP effector pathway. Biochemical analysis indicates that only one gene for adenylate cyclase must exist in yeast, since cells containing deletions of this gene do not produce cAMP. Surprisingly, however, complete deletions of the *CYR1* gene are not lethal. By tetrad analysis, approximately half of the spores without cyclase can germinate and give rise to very slow growing colonies (T. Toda et al., in prep.). We estimate that the doubling time of such cells is initially approximately 24 hours, which is in marked contrast to spores lacking *ras* function. These rarely germinate and, if they do, cease division soon after. We conclude from this that *ras* is likely to have a second function essential for yeast cells besides the stimulation of adenylate cyclase. Another line of experiments is consistent with this idea. Cell strains lacking the adenylate cyclase gene have been grown and transformed with a high-copy extrachromosomal plasmid containing the *RAS2*^{val19} gene. Such strains now evince a heat-shock phenotype and sensitivity to starvation.

The Regulatory Subunit of the cAMP-dependent Protein Kinase (BCY1). *bcy1* cells were first obtained as bypass mutants of cells with defective adenylate cyclase (Uno et al., *J. Biol. Chem. 257:* 14110 [1982]). Biochemically, *bcy1* cells appear to lack the regulatory subunit of the cAMP-dependent protein kinase. Phenotypically, *bcy1* cells, like *RAS2*^{val19} cells, are sensitive to nitrogen starvation and heat shock, presumably because they cannot arrest in G_1. *BCY1* was cloned from a centromere-linked library (constructed and generously supplied by M. Rose, Massachusetts Institute of Technology) by transforming *bcy1* cells with the library and selecting cells capable of resistance to nitrogen starvation and heat shock. We obtained one clone that displays all of the genetic features expected of a

BCY1 clone. Gene disruptions of *BCY1* were constructed and used to transform haploid yeast cells by gene replacement. The transformants display a phenotype similar to that of cells containing the spontaneously occurring *bcy1* mutation. In addition to the heat-shock phenotype, such cells do not grow at all on nonfermentable carbon sources or on galactose and grow poorly on glucose.

Sequence analysis confirms that *BCY1* encodes the regulatory subunit of the cAMP-dependent protein kinase. The primary amino acid sequence of the encoded protein is virtually identical with the primary amino acid sequence of the protein purified by E. Krebs and co-workers, which has been demonstrated to be the cAMP-dependent protein kinase of yeast. Moreover, M. Zoller (Genetics Section) has demonstrated that the *BCY1* protein expressed in *E. coli* can act as a cAMP regulatory subunit when combined with the bovine heart catalytic subunit of the cAMP-dependent protein kinase.

Catalytic Subunits of the cAMP-dependent Protein Kinase (TPK1, 2, *and* 3). While cloning genes that in high copy were able to suppress a temperature-sensitive, cell-division-cycle mutant called *cdc25*, we found a gene with the potential to encode a protein with great sequence similarity to the bovine heart cAMP-dependent protein kinase catalytic subunit. Originally called *CAK1*, in last year's Annual Report, we renamed this gene *TPK1*. Hybridization analysis indicated that *TPK1* was homologous to two related genes that we cloned and called *TPK2* and *TPK3*. Sequence analysis indicated that the three encode proteins that are nearly 90% homologous to each other and show greater than 50% homology with the bovine cAMP protein kinase. Biochemical analysis indicated that *TPK1* indeed encodes a catalytic subunit of the yeast cAMP-dependent protein kinase. *TPK2* and *TPK3* presumably also encode catalytic subunits, although we have not yet proved this. Consistent with this idea, when any of the three genes are present in high copy, they suppress the growth defect that otherwise results from disruption of the gene for adenylate cyclase.

The three *TPK* genes form a complementary triplet. Disruption of any pair has no readily discernible phenotype. Disruption of all three leads to cells that grow very slowly, with a doubling time that we estimate to be 24 hours. This phenotype is consistent with the phenotype of very slow growth that results from the disruption of the adenylate cyclase gene and differs from the phenotype of *ras*-disrupted cells that cannot grow at all. These results also suggest that *ras* has more effects on yeast than are mediated through the cAMP effector pathway.

High-affinity cAMP Phosphodiesterase (PDE2). A gene encoding a high-affinity cAMP phosphodiesterase, *PDE2*, was cloned by searching for genes that, in high copy, can reverse the heat-shock phenotypes of cells containing the *RAS2*^{val19} gene (see below). Sequence analysis of this gene showed that it encoded a protein with an amino acid composition similar to that of the high-affinity cAMP phosphodiesterase of bakers' yeast. Biochemical analysis of yeast cells containing disruptions of *PDE1* or expressing high levels of *PDE1* confirmed this conclusion. Comparison of the sequence of yeast *PDE1* protein with unpublished amino acid sequences of *Drosophila* and bovine cAMP phosphodiesterases indicated that there is a conserved 12-amino-acid sequence in all three.

SUPPRESSORS OF *RAS* FUNCTION IN *S. CEREVISIAE*

One fruitful approach to the genetics of *ras* is to examine genes that function either to bypass or to diminish *ras* function. Two different approaches can be taken. First, since *ras* genes are essential for growth in yeast cells, we can look for genes or mutations that relieve this requirement. Second, since yeast strains containing the *RAS2*^{val19} mutation are heat-shock-sensitive, we can look for genes or mutations that restore normal heat-shock sensitivity in such strains.

Genes That Do and Genes That Do Not Suppress Effects of the Loss of ras *Function.* We previously demonstrated that the loss of the functional *BCY1* gene, encoding the regulatory subunit of the cAMP-dependent protein kinase, renders cells resistant to the loss of adenylate cyclase or *ras* function. This result initially suggested that all the essential effects of *ras* are mediated through the adenylate cyclase system, a conclusion that we now know is wrong. To pursue this observation further, we conducted studies with the individual *TPK* genes, by transforming yeast cells with these genes on high-copy extrachromosomal replicating plasmids. We then examined the ability of such cells to lose adenylate cyclase or *ras* function. Although cells containing any of the *TPK* genes in high copy could grow read-

ily when they lose adenylate cyclase, the same is not true when they lose *ras*. These results suggested that, in addition to stimulating adenylate cyclase, *ras* proteins have other essential functions in yeast. We have since isolated two other genes (which we have provisionally named *SCH1* and *SCH9*) that can partially suppress the growth retardation that results from the loss of adenylate cyclase, but they do not suppress the lethality resulting from the loss of *ras*. These genes were obtained initially as suppressors of *cdc25* (see below).

Genes and Mutations That Suppress the RAS2^{val19} *Phenotype.* Strains containing the *RAS2*^{val19} mutation are sensitive to heat shock. We therefore sought genes in high-copy yeast libraries that could suppress the heat-shock phenotype of *RAS2*^{val19} strains. Two were found. One encoded the high-affinity cAMP phosphodiesterase, *PDE2*, which was described in a previous section. The other, which has been provisionally called *JUN1*, is of unknown function. *JUN1* has been sequenced but shows no homology with phosphodiesterases or other genes of known function. It encodes a 45,000-dalton protein, with two homologous domains of 10,000 daltons at its carboxyl terminus.

We also sought "suppressor" mutations in other genes that would render these cells heat-shock-resistant. Mutations in the *RAS2*^{val19} gene and mutations in the *CYR1* gene were encountered along with many others. One suppressor, *supC*, has been cloned and shown to be distinct from previously identified genes. Another of these suppressor mutations, called *supH*, is of particular interest. Cells with *supH* were found to be temperature-sensitive. Moreover, they were a-specific steriles. In a collaboration with the Laboratory, I. Herskowitz and coworkers (University of California at San Francisco) confirmed that *supH* was an a-specific-sterile and furthermore that it failed to make biologically active a-factor, although a-factor mRNA was made. Meantime, we showed that *supH* cells made *RAS2* proteins, but the adenylate cyclase of such cells behaved as though they lacked *ras* protein. It therefore seems likely that *supH* encodes a protein needed for processing both *ras* and a-factor. Sequence comparisons show that the a-factor gene encodes a protein with the terminal sequence Cys-*A*-*A*-*X*, where *A* is any aliphatic amino acid and *X* is the terminal amino acid. This site is probably the site for fatty acid acylation and is required for the proper functioning of *ras* proteins. It is therefore possible that *supH* encodes the enzyme that performs this acylation.

SUPH has been cloned and is now being characterized. The genetic properties of *supH* are themselves of great interest. Although high-copy plasmids expressing *RAS2* will suppress the temperature-sensitive defect of *supH*, high-copy plasmids of *TPK1* will not. Since high-copy plasmids of *TPK1* will suppress the growth defect of *ras*$^-$ cells in an otherwise wild-type background, *supH* must cause defects in yet other pathways not controlled by *ras* but which nevertheless interact with *ras*-controlled pathways. From our studies, we infer that there are at least three essential pathways controlling growth in *S. cerevisiae*. The first, the cAMP pathway, is regulated by *ras* proteins. A second, which we are provisionally calling the "*ras* alternative" pathway, is also controlled by *ras*. It is clear that under certain conditions, the first pathway can subsume the essential functions of the second. Genetic data, not presented here, suggest that these two pathways may coordinately control certain cellular phenotypes. A third pathway is not regulated by *ras* but must cooperate with the *ras* alternate pathway in some manner. All three pathways are dependent on a functional *SUPH* gene.

Since *supH* is likely to cause defects in the modification of proteins with the Cys-*A*-*A*-*X* terminal sequence, some proteins of that class are likely to be regulatory proteins in yeast. *supH* may provide a genetic and biochemical entree to such proteins. Moreover, we have not excluded the possibility that *SUPH* itself encodes a regulatory protein, controlling the activity of *ras* and other proteins.

OTHER RELATED STUDIES

In addition to the above studies, which have mainly centered on the *ras*/adenylate cyclase pathways, our laboratory has been studying the cell-division-cycle mutant *cdc25* in collaboration with M. Zoller and L. Levin. *cdc35* (which is allelic to *CYR1*) and *cdc25* are the two temperature-sensitive mutants that comprise the first group of start mutants, i.e., mutants that arrest in G_1 prior to the sex factor G_1 arrest point. We began by isolating genes that in high copy could suppress the growth arrest of *cdc25*. Two genes were initially isolated. The *TPK1* gene, formerly called *CAK1*, was one. It turned out to be one of the cAMP-dependent protein kinase catalytic subunits and has been described in a previous

section. *TPK1* is not allelic to *cdc25*. The second suppressor gene was shown to be allelic and complementary to *cdc25* and was therefore the *CDC25* gene itself. The nucleotide sequence of *CDC25*, completed by L. Levin, indicates a potential to encode a large protein of 185,000 daltons, with no similarity to other known proteins.

We have investigated genes that in high copy suppress the *cdc25* temperature-sensitive allele or more severely disrupted *CDC25* alleles. Most genes of the known *ras*/adenylate cyclase pathway appear to be capable of suppressing both: high-copy $RAS2^{val19}$, *CYR1*, *TPK1*, *TPK2*, and *TPK3*. High-copy *RAS2* wild type will suppress the *cdc25* temperature-sensitive allele but not the more severely disrupted alleles of *CDC25*. In addition, we have isolated two new genes, *SCH1* and *SCH9*, by screening high-copy libraries that suppress the temperature-sensitive allele. These genes do not suppress the more severely disrupted alleles. More will be said about these genes below.

These data suggest that the *CDC25* pathway and adenylate cyclase overlap in some manner. One possibility is that *CDC25* is a component of the adenylate cyclase pathway. However, biochemical experiments indicate that cells with the *CDC25* disruption have normal adenylate cyclase. These results argue, but do not prove, that the *CDC25* pathway and the *CYR1* pathway are distinct and that high-level expression of the cAMP effector pathway can suppress the need for the *CDC25* pathway, perhaps in a manner analogous to its suppression of the need for the *ras* alternate pathway. Thus, we should seriously consider whether *CDC25* is part of the *ras* alternate pathway.

The genes *SCH1* and *SCH9*, initially isolated because they suppressed the temperature-sensitive defect of *cdc25* cells, are each of considerable interest. Both genes can suppress the growth defects of cells that lack adenylate cyclase. Therefore, they may be parts of pathways with the capacity to assume the functions of the cAMP effector pathway. The striking picture that emerges is that there are multiple pathways in *S. cerevisiae* with potentially overlapping domains of command.

TABLE 1 Genes of *S. cerevisiae* That Interact with the *ras*/cyclase Pathway

Gene	Identity or identifying properties
RAS1	activates adenylate cyclase
RAS2	activates adenylate cyclase
CYR1	adenylate cyclase
BCY1	regulatory subunit of cAMP-dependent protein kinase
TPK1	catalytic subunit of cAMP-dependent protein kinase
TPK2	catalytic subunit of cAMP-dependent protein kinase
TPK3	catalytic subunit of cAMP-dependent protein kinase
PDE1	high-affinity cAMP phosphodiesterase
JUN1	suppressor of the $RAS2^{val19}$ phenotype
SUPH	involved in functional modification of *ras* proteins and a-factor
CDC25	mutants in *CDC25* are suppressed by genes of the *ras*/cAMP effector pathway
SCH1	suppressor of *cdc25* and *cyr1*
SCH9	suppressor of *cdc25* and *cyr1*

CONCLUSIONS

Table 1 summarizes the genes we have cloned and characterized that interact with the yeast *ras*/cyclase pathways. The overwhelming impression from our studies is that control of growth in *S. cerevisiae* is complex. There are probably multiple regulatory pathways that in some cases not only can act coordinately, but also can compensate for each other. The complexity of growth control in *S. cerevisiae* will probably rival the complexity now being seen in animal cells and may provide a good model for the latter. What is the need for this complexity? One simple answer is possible. The growth phase of any cell requires the production of cellular constituents in proper proportions and at rates compatible with available nutrients. Some of this control can of course be accomplished by "short" feedback loops. For example, attaining the proper proportion of membrane phospholipids could easily be regulated by such short feedback loops. But how are overall rates of membrane synthesis determined and coordinated, for example, with rates of protein synthesis? Surely, these larger lines of production must be as closely regulated as, say, the decision to begin DNA replication. The growth effector pathways may therefore control such lines of production and be integrated by feedback control from critical substrates of these production lines. Perturbations of any one pathway, for example, by mutating or overexpressing critical controlling proteins, may therefore perturb the level of activity of the other pathways.

Mammalian Oncogenes

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We have maintained our interest in the growth control of mammalian cells by studying mammalian oncogenes. Our studies on mammalian *ras* have taken clues from the study of *ras* in yeast. We have developed a sensitive in vivo bioassay for *ras* proteins: the induction of frog oocyte maturation. We have shown that *ras* proteins probably do not effect adenylate cyclase in the frog oocyte but rather exert strong effects through other pathways. In addition, we are studying two human oncogenes we have recently isolated: the *ros1* and *mas1* genes. These genes were isolated using a modification of the NIH-3T3 transfection assay, which identifies oncogenes by their ability to induce tumorigenicity (Fasano et al., *Mol. Cell. Biol. 4:* 1695 [1984]). It is now clear that this assay identifies normal human genes that have the potential to act as oncogenes when amplified, overexpressed, or rearranged as a secondary consequence of DNA transfer (C. Birchmeier et al., submitted; D. Young et al., submitted).

MAMMALIAN *ras* GENES

Our studies of *Saccharomyces cerevisiae* prompted us to test whether *ras* proteins can effect adenylate cyclase in vertebrate cell systems. For this purpose, we used a frog oocyte microinjection system. This system was chosen because it was known that frog oocyte maturation is dependent on the activity of its cAMP effector pathway and because it is possible to perform biochemical experiments on small numbers of microinjected eggs. We have found that purified mammalian Ha-*ras* proteins, prepared from an *Escherichia coli* expression system (Gross et al., *Mol. Cell. Biol. 5:* 1015 [1985]) can induce immature oocytes to progress from prophase to metaphase (Birchmeier et al., *Cell 43:* 615 [1985]). The Ha-ras^{val12} is perhaps 100-fold more potent than the normal Ha-ras^{gly12} in inducing oocyte maturation. We have observed no significant changes in cAMP production in oocytes due to injection of mutant Ha-*ras* protein. Moreover, the *ras*-induced effects are only partly blocked by cholera toxin, an agent that totally blocks the maturation effects of progesterone by raising cAMP production. We conclude from this that the yeast *RAS* responsive systems are not necessarily functionally analogous to the vertebrate *ras* responsive systems. Nevertheless, it is clear that *ras*-induced frog oocyte maturation provides an excellent model system for testing ideas of *ras* protein function.

THE HUMAN *ros1* GENE

Last year, we described the isolation of two new human oncogenes called *mcf2* and *mcf3*, which were obtained using the DNA cotransfer and tumorigenicity assay (Fasano et al., *Cell. Biol. 4:* 1695 [1984]). Nucleotide sequence analysis of *mcf3* cDNA clones indicated that the *mcf3* gene arose by rearrangement of a human gene homologous to the viral v-*ros* gene, which we have called *ros1*. It is likely that this rearrangement occurred during or after DNA transfer and was responsible for activating the oncogenic potential of *ros1*. This gene, like its v-*ros* counterpart, contains a transmembrane domain and a carboxyterminal domain that is homologous to the known tyrosine protein kinases. The rearrangement creating *mcf3* resulted in the loss of a putative extracellular domain. It is likely that the normal *ros1* gene, like the normal counterpart of the viral v-*erb* and v-*fms* genes, is a growth factor or hormone receptor. We are currently attempting to obtain a full-length cDNA clone to help us identify the extracellular domain and the physiologic ligand of this receptor. Expression studies in progress clearly indicate that the *ros1* gene is expressed in a high proportion of human tumor cell lines and hence might be a useful tumor cell marker.

THE HUMAN *mas1* GENE

We have begun the characterization of another human oncogene that we have called *mas1*. This gene was isolated using the same methodology used for isolating the *mcf3* gene described above (D. Young et al., submitted). Comparison of the transforming locus with the placental locus, cDNA cloning and sequencing, and S1-nuclease protection experiments have led to the following conclusions. Like *mcf3*, *mas1* was activated in NIH-3T3 cells during or after gene transfer by a DNA rearrangement, and, like *mcf3*, there is no evidence that *mas1* was activated in the tumor cells from which it ultimately derived. Unlike *mcf3*, the transforming *mas1* gene

is not rearranged within coding regions. Rather, its ability to transform cells appears to be entirely related to its high level of expression, a consequence of a rearrangement involving DNA 5′ to coding sequences. The normal *mas1* gene, cloned from a human placental cosmid library, is only weakly transforming.

Two aspects of the *mas1* gene make it of considerable interest as an oncogene. First, NIH-3T3 cells transformed with *mas1* are highly tumorigenic and grow to high saturation densities but appear to be morphologically indistinguishable from normal NIH-3T3 cells. Second, the protein encoded by *mas1* is very hydrophobic. In its hydropathy profile, it closely resembles the visual rhodopsins and is predicted to have seven transmembrane domains. In this respect, it is novel among cellular oncogenes. Our preferred hypothesis is that the *mas1* protein, like rhodopsin, is a signal-transducing receptor that activates a GTP-binding protein. An understanding of the mechanism whereby *mas1* transforms cells is likely to lead to a new insight into growth control mechanisms.

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