GENETICS OF CELL PROLIFERATION

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Our laboratory studies the relationship among oncogenes, signal transduction, and malignant transformation, with particular emphasis on *RAS* genes. Three mammalian *RAS* genes, Ha-*ras*, Ki-*ras*, and N-*ras*, are capable of the malignant transformation of cultured animal cells. Mutations in these genes have been linked to a large number of human cancers. Perhaps as much as 25% of human tumors contain mutant *RAS* genes, pointing to a common metabolic defect in a large fraction of human malignancy. It is therefore imperative that we understand the biochemical function of *RAS* proteins.

The *RAS* genes encode closely related proteins that bind guanine nucleotides (Scolnick et al., *Proc. Natl. Acad. Sci. 76:* 5355 [1979]; Shih et al., *Nature 287:* 686 [1980]); Ellis et al., *Nature 292:* 506 [1981]) and are localized to the inner surface of the plasma membrane (Willingham et al., *Cell 19:* 1005 [1980]; Papageorge et al., *J. Virol. 44:* 509 [1982]). Normal *RAS* proteins also slowly hydrolyze GTP (Gibbs et al., *Proc. Natl. Acad. Sci. 81:* 5704 [1984]; McGrath et al., *Nature 310:* 644 [1984]; Sweet et al., *Nature 311:* 273 [1984]). These properties are similar to those of the G proteins, which has led to the widespread

expectation that *RAS* proteins, like G proteins, are involved in the transduction of membrane signals that are linked to cellular proliferation or differentiation. Many of the mutations that activate the *RAS* genes result in the production of proteins with impaired GTP hydrolysis (Gibbs et al., *Proc. Natl. Acad. Sci. 81:* 5704 [1984]; McGrath et al., *Nature 310:* 644 [1984]; Sweet et al., *Nature 311:* 273 [1984]). This has suggested that, like G proteins, *RAS* proteins are active when bound to GTP but inactive when bound to GDP. The biochemical function of the mammalian *ras* proteins is unknown.

We have been studying the function of the yeast *RAS* genes in the expectation that such study will lead to insights into the functioning of the mammalian *RAS* genes. *Saccharomyces cerevisiae* have two genes, *RAS1* and *RAS2*, that are structurally homologous to the mammalian *RAS* genes (DeFeo-Jones et al., *Nature 306:* 707 [1983]; Dhar et al., *Nucleic Acids Res. 12:* 3611 [1984]; Powers et al., *Cell 36:* 607 [1984]). The yeast and mammalian *RAS* genes are functionally related as well, since mammalian *RAS* genes can complement yeast lacking their endogenous *RAS* genes (Kataoka et al., *Cell 40:* 19 [1985]), and yeast *RAS* genes can malignantly transform cultured animal cells (DeFeo-Jones et al., *Science 228:* 179 [1985]). In the yeast *S. cerevisiae, RAS* proteins appear to control events related to growth arrest. The *RAS2* gene can be activated by a point mutation analogous to the point mutation of Ha-*ras*, which activates its oncogenic potential (Kataoka et al., *Cell 37:* 437 [1984]). Cells carrying the activated $RAS2^{val19}$ gene fail to arrest in G_1 when starved, remain heat-shock-sensitive when they reach stationary phase, and fail to accumulate storage carbohydrates (Kataoka et al., *Cell 37:* 437 [1984]; Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]). These same sets of phenotypes are observed when the adenylyl cyclase pathway is activated (Uno et al., *J. Biol. Chem. 257:* 14110 [1982]) and first led us to suspect an interaction between *RAS* proteins and adenylyl cyclase.

Our laboratory is also continuing investigations into two mammalian oncogenes. The *MAS* oncogene encodes a potential hormone or neurotransmitter receptor with seven transmembrane domains. The overexpression of *MAS* leads to a minimally transformed phenotype. The *ROS* oncogene encodes a large transmembrane tyrosine kinase, which also is a potential receptor for an unknown ligand. The *ROS* gene is expressed and rearranged in some glioblastomas.

Interaction between *RAS* Proteins and Yeast Adenylyl Cyclase

J. Field, J. Colicelli, R. Ballester, T. Michaeli

In yeast, *RAS* proteins are required for the proper functioning of adenylyl cyclase (Toda et al., *Cell 40:* 27 [1985]). This is readily seen both from studies in vivo with mutant yeast strains (Toda et al., *Cell 40:* 27 [1985]; Nikawa et al., *Genes Dev. 1:* 931 [1987]) and from studies in vitro (Broek et al., *Cell 41:* 763 [1985]; Field et al., *Mol. Cell. Biol. 72:* 2128 [1987]; Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). Our in vitro systems use *RAS* proteins purified from an *Escherichia coli* expression system (Broek et al., *Cell 41:* 763 [1985]; Gross et al., *Mol. Cell. Biol. 5:* 1015 [1985]) and an adenylyl cyclase complex purified from *S. cerevisiae* (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). Our method of purification of adenylyl cyclase is novel. It involves making in yeast a fusion protein of adenylyl cyclase with a small amino-terminal peptide epitope. Extracts of yeast are passed over an affinity column containing monoclonal antibodies directed against the peptide epitope. Adenylyl cyclase is then eluted with synthetic peptide, resulting in a greater than 100-fold purification. The resulting complex contains a 70-kD component that copurifies with adenylyl cyclase activity in glycerol sedimentation gradients.

We conclude from our in vitro studies that *RAS* proteins interact directly with the adenylyl cyclase complex. The addition of either yeast *RAS2* protein or mammalian Ha-*ras* protein can result in a greater than 20-fold stimulation of activity. We can also conclude from in vitro work that *RAS* proteins bound to GTP stimulate adenylyl cyclase, but *RAS* proteins bound to GDP do not (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). Thus, the activity of *RAS* proteins is controlled by the guanine nucleotide they bind, consistent with the model of oncogenesis proposed for mutant, activated *RAS*. We have also concluded from our work that the stimulation of adenylyl cyclase requires the continued presence of *RAS* proteins.

We cannot conclude from our work that *RAS* proteins act directly on adenylyl cyclase itself, since the adenylyl cyclase complex copurifies with a 70-kD protein (Field et al., *Mol. Cell. Biol. 8:* 2159 [1988]). We are currently investigating the role of the 70-kD protein in the *RAS* responsiveness of adenylyl cyclase (1) by attempting the purification of the 70-kD protein and cloning the gene that encodes it and

(2) by performing deletion analysis of adenylyl cyclase.

Control of *RAS* Protein Activity

S. Powers, D. Broek, S. Cameron, K. Ferguson, K. O'Neill

In *S. cerevisiae*, the *CDC25* protein appears to control *RAS* protein activity. *cdc25*^{ts} alleles were first discovered as cell-cycle G_1 arrest mutants (Hartwell et al., *Genetics 74:* 267 [1973]; Hartwell, *Bacteriol. Rev. 38:* 164 [1974]). Cells lacking *cdc25* are deficient in cAMP and have aberrant adenylyl cyclase activity (Martegani et al., *EMBO J. 5:* 375 [1986]; Broek et al., *Cell 48:* 789 [1987]; Camonis et al., *EMBO J. 5:* 375 [1986]; Nikawa et al., *Genes Dev. 1:* 931 [1987]). Cells that contain the activated, mutant RAS2^{val19} gene do not require the *CDC25* product (Broek et al., *Cell 48:* 789 [1987]; Robinson et al., *Science 235:* 1218 [1987]). These data are consistent with a model in which the *CDC25* product acts upstream of *RAS* and causes its activation, possibly by catalyzing nucleotide exchange.

Further evidence in favor of this model has come from our discovery of mutant *RAS* proteins that behave as though they interfere with *CDC25* activity (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]). These mutant *RAS* genes were found in the course of a genetic screen for temperature-sensitive *RAS* mutants. We found in this screen dominant temperature-sensitive lethal *RAS2* alleles. Significantly, lethality can be overcome by the presence of the *CDC25* gene on a high-copy plasmid but only if a wild-type *RAS2* or *RAS1* gene is also present. Lethality can also be overcome if cells contain the mutationally activated *RAS2*^{val19} gene. Thus, the mutant *RAS* proteins appear to interfere with the activation of wild-type *RAS* proteins, perhaps by forming a complex with *CDC25* proteins. The mutations in interfering *RAS* genes localize to the region that encodes part of a consensus nucleotide-binding site common to many GTP-binding proteins (Powers et al., *Mol. Cell. Biol. 9:* 390 [1989]).

To explain our results with *CDC25* and *RAS*, we propose that *RAS* proteins and *CDC25* proteins normally undergo a transient and direct interaction, similar to models that have been proposed to explain the interaction of receptors with G proteins (Gilman, *Ann. Rev. Biochem. 56:* 615 [1987]; Stryer, *Ann. Rev. Neurosci. 9:* 87 [1986]). As in those models, *CDC25* proteins interact with the GDP-bound form of *RAS* proteins and, by virtue of stabilizing the transitional state of nucleotide-free *RAS* protein, catalyze nucleotide exchange. We propose that the dominant temperature-sensitive *RAS* proteins remain bound to *CDC25* protein because alterations in the consensus nucleotide-binding site alter nucleotide affinity and stabilize a nucleotide-free *RAS-CDC25* protein complex.

Interfering Mutants in Signal Transduction Pathways

S. Powers, T. Michaeli, J. Field, J. Colicelli, R. Ballester

The discovery that there exist mutant forms of *RAS* that interfere with activation of normal *RAS* led us to think about interfering mutants in a more general sense. In the broadest possible terms, if there is a signal transduction pathway wherein protein X interacts with protein Y, which then interacts with protein Z in a cascade of information flow, one can expect at least four types of dominant interfering mutant proteins: mutants of X that complex ineffectively with Y; mutants of Y that complex ineffectively with X; mutants of Y that complex ineffectively with Z; and mutants of Z that complex ineffectively with Y. Genetic screens can be designed to search for mutations that produce these kinds of proteins, and such mutants may be valuable tools in the analysis of complex signaling pathways.

We have applied this approach to the *RAS*/adenylyl cyclase pathway of *S. cerevisiae*. We randomly mutagenized Ha-*ras* genes by passage of plasmids carrying Ha-*ras* through a mutator strain of *E. coli* (Silhavy et al., in *Experiments with gene fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York [1984]), and screened the mutagenized plasmids for their ability to suppress the heat-shock sensitivity of strains of yeast carrying the *RAS2*^{val19} gene. One such mutant was found, and sequence analysis revealed that it contained an arginine for cysteine substitution at codon 186 (T. Michaeli, submitted). This disrupts the Cys-*A*-*A*-*X* (where *A* is any aliphatic amino acid and *X* is the terminal amino acid) consensus sequence of *RAS* proteins (Taparowsky et al., *Cell 34:* 581 [1983]; Powers et al., *Cell 36:* 607 [1984]) that functions as a target for the fatty acid addition which causes membrane localization

of *RAS* (Willumsen et al., *Nature 310:* 583 [1984]; Powers et al., *Cell 47:* 413 [1986]). We found that other mutations in this region which destroy the consensus sequence also result in Ha-*ras* genes that interfere with the phenotype of $RAS2^{val19}$.

Our analysis of the mutant Ha-*ras* proteins with a disrupted Cys-*A-A-X* consensus sequence indicates an unexpected complexity of *RAS* interactions. The mutant proteins remain cytosolic, in keeping with the findings of other investigators that the Cys-*A-A-X* sequence is required for membrane localization (Willumsen et al., *Nature 310:* 583 [1984]). The Ha-*ras* mutants do not block $RAS2^{val19}$ protein from localizing to the membrane. Their effect is therefore not likely to be due to dominant effects on *RAS* protein processing. Competition experiments indicate that the effects of the Ha-*ras* mutants are competed by overexpression of $RAS2^{val19}$ but not by overexpression of *CYR1*, the gene that encodes adenylyl cyclase. From this, we conclude that these Ha-*ras* mutant proteins interfere with a cytosolic factor which may facilitate the interaction of *RAS2* with adenylyl cyclase. Alternatively, the mutant Ha-*ras* may interfere with a second function of *RAS*. Evidence for multiple functions of *RAS* in yeast is given below.

We have also found mutant *CYR1* genes that interfere with the phenotypes of $RAS2^{val19}$. We used a strategy similar to the one described above. We passaged a plasmid carrying *CYR1* through a mutator strain of *E. coli* and readily found clones of *CYR1* that blocked the heat-shock sensitivity of $RAS2^{val19}$ strains. The ease with which this screen yielded interfering mutations in *CYR1* led us to suspect that virtually any mutation that disrupted the enzymatic function of adenylyl cyclase could result in an interfering protein. Direct tests proved this hypothesis to be correct. The region encoding the catalytic portion of the adenylyl cyclase is located at the 3′ end of *CYR1* (Kataoka et al., *Cell 43:* 493 [1985]). Frameshift or deletion mutations in this region result in the production of interfering forms of the *CYR1* product. Competition assays suggest that the effects of defective *CYR1* genes can be suppressed by overexpression of *RAS* proteins. It is likely, we think, that the mutant *CYR1* genes encode proteins that form ineffective complexes with *RAS* proteins. More generally, it may be true that proteins which are the targets of *RAS* action can interfere with *RAS* function when they are functionally incompetent.

Feedback Regulation of RAS Activity

S. Cameron

In the course of screens for genes that, when overexpressed, can suppress the phenotypes induced by $RAS2^{val19}$, we cloned two genes of *S. cerevisiae* that encode cAMP phosphodiesterases *PDE1* and *PDE2* (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]; Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]). Together, these genes appear to encode the totality of cAMP phosphodiesterase activity measurable in yeast cell extracts (Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]). Surprisingly, we found that cells which lacked these genes, but that were otherwise normal, did not accumulate enormous levels of cAMP (Nikawa et al., *Genes Dev. 1:* 931 [1987]). One explanation for this result is that elevated levels of cAMP directly or indirectly feedback to turn off the further production of cAMP. Confirmation of this theory comes from examining cAMP levels in cells that lack the *PDE* genes but contain the $RAS2^{val19}$ gene (Nikawa et al., *Genes Dev. 1:* 931 [1987]). Such cells have enormously elevated levels (over 1000-fold!) of cAMP. In addition to confirming the existence of feedback, these studies indicate that the $RAS2^{val19}$ protein is unresponsive to feedback controls.

Feedback requires the activity of the cAMP-dependent protein kinases (cAPK). The catalytic subunits of these genes are named *TPK1*, *TPK2*, and *TPK3* (Toda et al., *Cell 50:* 277 [1987]). Cells with attenuated *TPK* genes have enormously elevated cAMP levels when they are grown in rich medium containing glucose (Nikawa et al., *Genes Dev. 1:* 931 [1987]). This result is consistent with the idea that, through homeostasis, the activity of the cAPK regulates cAMP levels. A dramatic demonstration of this is seen upon feeding glucose to cells with an attenuated cAPK system. Wild-type cells show a biphasic response to glucose when fed: A tenfold elevation of cAMP ensues within minutes and diminishes to basal levels within 10 minutes (Beullens et al., *Eur. J. Biochem. 172:* 227 [1988]). In cells with an attenuated cAPK system, glucose feeding does not induce a biphasic response in cAMP levels. Rather, there is a sharp, perhaps 100-fold, elevation of cAMP levels that remain elevated as long as glucose is present (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). This result indicates that (1) the

cAPK system participates in the physiologic feedback regulation of the cAMP response to glucose and (2) glucose is a stimulant of the system, and the system remains stimulated as long as glucose is present. These results on feedback of cAMP production also suggest the possibility that the levels of cAMP in a cell may oscillate in response to cycles of stimulation and feedback.

RAS-independent Pathways for Growth Control

S. Cameron, J. Colicelli, A. Vojtek, K. Ferguson, H. Xu

The yeast *S. cerevisiae* undergoes marked changes in response to nutrient limitation. Diploid cells will sporulate under appropriate starvation conditions. Haploid cells respond by becoming heat-shock-resistant and by accumulating storage carbohydrates. Since these responses can be mimicked by mutations that lower the activity of the cAMP-dependent protein kinase (cAPK) and can be blocked by mutations that raise the activity of the cAPK, it is natural to assume that physiologic modulation of cAMP activity by cAMP regulates these responses. However, the cAPK system may not be the only signaling system that generally regulates growth and responses to nutrition. We have used the genetics available in yeast to examine this question more directly.

The yeast *S. cerevisiae* contains a cAMP-responsive kinase activity. Genes encoding a regulatory subunit, *BCY1* (Toda et al., *Mol. Cell. Biol. 7:* 1371 [1987]), and three catalytic subunits, *TPK1*, *TPK2*, and *TPK3* (Toda et al., *Cell 50:* 277 [1987]), have been isolated. Disruption of the *BCY1* gene results in a very severe phenotype (Toda et al., *Mol. Cell. Biol. 7:* 1371 [1987]). We have characterized the role of the cAPK catalytic subunit genes in producing the *bcy1*$^-$ phenotype, and in the process, we have generated mutant cAPK catalytic subunit genes (*TPK*) that suppress the *bcy1*$^-$ defects (Cameron et al., *Cell 53:* 555 [1988]). The mutant *TPK* genes appear to encode functionally attenuated catalytic subunits of the cAPK. *bcy1*$^-$ yeast strains containing the mutant *TPK* genes respond appropriately to nutrient conditions, even in the absence of *CDC25*, both *RAS* genes, or *CYR1*. Together, these latter genes encode the known components of the cAMP-generating machinery. The results indicate that cAMP-independent mechanisms must exist for regulating glycogen accumulation, sporulation, and the acquisition of thermotolerance in *S. cerevisiae*. In particular, *RAS*-independent signaling systems must exist. Indeed, we have isolated many genes that can modulate the phenotype of the activated *RAS*/cAMP signaling system but that do not appear to belong to that signaling system.

Evidence for Additional Functions of *RAS*

S. Powers, T. Michaeli, A. Vojtek

Most of the effects of *RAS* on yeast cells can be explained by their action on adenylyl cyclase. The phenotype of cells containing *RAS2*^{val19} can readily be understood as a consequence of the perturbation of cAMP production: cAMP levels are elevated in cells containing *RAS2*^{val19}; activation of the cAMP-dependent protein kinases leads to a phenotype that closely resembles that due to *RAS2*^{val19}; and elevated expression of cAMP phosphodiesterases reverses the *RAS2*^{val19} phenotype (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]; Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]). Moreover, the lethality that otherwise results from disruption of both *RAS* genes can be overcome by disruption of the gene, *BCY1*, that encodes the cAMP-dependent protein kinase regulatory subunit (Toda et al., *Cell 50:* 277 [1987]). The resulting unbridled protein kinase activity is sufficient to complement the loss of *RAS* function. However, there are subtle effects of disruption of both the *RAS1* and *RAS2* genes that do not appear to be identical to the effects of disrupting the adenylyl cyclase gene, and mutations in *RAS* act in ways that cannot be explained readily by effects upon adenylyl cyclase.

There are two major differences between cells lacking *RAS* and cells lacking *CYR1*. First, haploid spores that lack the *CYR1* gene are often viable, although they give rise to very slow growing colonies, whereas haploid spores that lack both *RAS1* and *RAS2* genes are almost never viable (Toda et al., in *Oncogenes and cancer*, Japan Sci. Soc. Press, Tokyo/VNU Sci Press [1987]). Second, overexpressing the *TPK* genes can readily suppress the growth defect resulting from lack of *CYR1*, but cannot so readily suppress the growth defects resulting from lack of *RAS* genes (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). Indeed, such

RAS-deficient strains suppressed by *TPK* genes are often temperature-sensitive. Thus, it appears that *RAS* may have additional functions besides the stimulation of adenylyl cyclase.

The results described above can each be explained in many ways. For example, one may propose that there is a second gene encoding adenylyl cyclase. We have rigorously eliminated this possibility. If such a second adenylyl cyclase existed, it would have to produce 1000-fold lower cAMP levels than the *CYR1* gene. Alternatively, one can propose that it is better to have no cAMP (*cyr1*$^-$) than a little (*ras1*$^-$ *ras2*$^-$). However, there is one telling piece of evidence that rules out this possibility. We have shown that many strains that lack both *CYR1* and *RAS*, but that are viable because *TPK* genes are highly expressed, are temperature-sensitive. This temperature sensitivity is cured by expressing *RAS* in such cells. Thus, *RAS* can act even in the absence of adenylyl cyclase. Lack of *CDC25* function produces the same defects as lack of *RAS* function even in the absence of *CYR1*. Hence, we conclude that *RAS* has additional functions besides stimulating adenylyl cyclase and that these functions are also shared by *CDC25* (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]).

Comparison of Mammalian and Yeast RAS

S. Powers, J. Field, K. Ferguson, R. Ballester, T. Michaeli, J. Colicelli

The similarities of the mammalian and yeast *RAS* genes are striking. The mammalian Ha-*ras* can complement yeast lacking their own *RAS* genes. Purified Ha-*ras* protein can stimulate purified yeast adenylyl cyclase (Broek et al., *Cell 41:* 763 [1985]). Genetic experiments demonstrate that Ha-*ras* can provide the additional functions of *RAS* in yeast, complementing the loss of *RAS* even in strains that lack adenylyl cyclase (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]).

There are other similarities between mammalian and yeast *RAS*. Mammalian Ha-*ras* protein, like yeast *RAS*, is probably subject to feedback inhibition (Bar-Sagi and Feramisco, *Science 233:* 1061 [1986]). We have evidence, too, that Ha-*ras* can interact with *CDC25*. The analogous mutations can be introduced into Ha-*ras*, which cause the dominant interfering mutants of *RAS2*. When these mutant Ha-*ras* genes are expressed in yeast, they also appear to block *CDC25* activity (Powers et al., *Mol. Cell.Biol. 9:* 390 [1988]). Similar mutants are also interfering in animal cells (Feig and Cooper, *Mol. Cell. Biol. 8:* 3235 [1988]), suggesting that there is a mammalian protein that catalyzes nucleotide exchange in mammalian *RAS* proteins. Perhaps there is a *CDC25* homolog in mammals.

Two other questions of similarity are raised by our studies of yeast. First, in yeast, *RAS* absolutely controls its effector pathway. It is not clear if this is so in mammals, although we suspect it is so. Second, in yeast, it is likely that *RAS* has more than one function. It is quite possible that *RAS* proteins also have more than one function in mammalian cells. This might explain some of the difficulty of assigning a function to mammalian *RAS*.

There are obvious differences between mammalian and yeast *RAS* proteins. The most glaring difference appears to be in the immediate biochemical function of the *RAS* proteins in their respective hosts. It is unlikely that mammalian *RAS* functions to stimulate adenylyl cyclase in vertebrates (Birchmeier et al., *Cell 43:* 615 [1985]). Indeed, a fundamentally different model of *RAS* action has been proposed in mammalian cells. Our model of *RAS* action is rather like the model of action for G proteins and transducin (Gilman, *Ann. Rev. Biochem. 56:* 615 [1987]). This model is strongly supported by experiments in yeast. A radically different model, emerging from the discovery of a *G*TPase-*a*ctivating *p*rotein (GAP), and speculative analogies between *RAS* proteins and bacterial elongation factor EF-Tu, has been proposed (Adari et al., *Science 240:* 518 [1988]; Cales et al., *Nature 332:* 548 [1988]). Our own opinion is that the latter model is wrong. There are too many similarities between yeast and mammalian *RAS* to cause us to abandon the yeast model. Our own studies point to the complexity of *RAS* interactions with its effectors, and although the identities of the individual effectors may have evolved during speciation, we feel that the patterns of *RAS* interactions may have changed little in evolution.

The Search for the Mammalian *RAS* Target

J. Collicelli, T. Michaeli, C. Birchmeier, J. Field

Our discoveries of interfering mutants in the *RAS* signaling pathway have led to a strategy for searching for the target of *RAS* protein action in mammalian cells. We have postulated that the function of *RAS*

in yeast would be inhibited by the expression, in yeast, of the mammalian target for *RAS* action. We have therefore constructed rat brain cDNA libraries in yeast expression vectors to test this idea. In a pilot experiment, we transformed yeast containing the activated $RAS2^{val19}$ gene and screened for heat-shock-resistant colonies among the transformants. One colony was identified that contained a vector that reproducibly induced heat-shock resistance in yeast strains carrying $RAS2^{val19}$. Analysis revealed that this vector contained a cDNA insert with the potential to encode a protein highly homologous to the *Drosophila dunce* product (Chen et al., *Proc. Natl. Acad. Sci. 83:* 9313 [1986]), and we tentatively call our gene *DNC* (Colicelli et al., *Proc. Natl. Acad. Sci.* [1989] in press).

The *dunce* locus was identified in mutant flies with defects in learning (Chen et al., *Proc. Natl. Acad. Sci. 83:* 9313 [1986]). *dunce* encodes a cAMP phosphodiesterase, leading us to suspect that the mammalian gene we cloned also encoded a cAMP phosphodiesterase. To test this idea, we transformed a yeast strain that lacks the two genes which encode cAMP phosphodiesterases, *PDE1* (Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]) and *PDE2* (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]). $pde1^{-}$ $pde2^{-}$ yeast carrying the rat *DNC* express a high-affinity cAMP phosphodiesterase (Colicelli et al., *Proc. Natl. Acad. Sci.* [1988] in press). We do not believe *DNC* encodes the mammalian *RAS* target. Rather, it is likely that *DNC* suppresses $RAS2^{val19}$ by lowering cAMP levels. As precedent, overexpression of either *PDE* gene suppresses $RAS2^{val19}$ (Sass et al., *Proc. Natl. Acad. Sci. 83:* 9303 [1986]; Nikawa et al., *Mol. Cell. Biol. 7:* 3629 [1987]).

These results have import for the study of cAMP phosphodiesterase activity in mammals. First, we have now identified a strategy useful for cloning mammalian cAMP phosphodiesterases. This may lead to the discovery of a wide family of enzymes of great physiological importance. Second, once cloned, the biochemical and pharmacological properties of mammalian phosphodiesterases may be studied after their expression in yeast lacking their own endogenous phosphodiesterases.

The *ROS* Oncogene

C. Birchmeier, S. Sharma, K. O'Neill, L. Rodgers

As reported previously, we have identified oncogenic forms of the human *ROS* gene (Fasano et al., *Mol. Cell. Biol. 4:* 1695 [1984]; Birchmeier et al., *Mol. Cell. Biol. 6:* 3109 [1986]). This gene has the potential to encode a transmembrane tyrosine kinase (Neckameyer and Wang, *J. Virol. 53:* 879 [1985]; Birchmeier et al., *Mol. Cell. Biol. 6:* 3109 [1986]). Like other oncogenes in this family, *ROS* probably encodes a membrane receptor/kinase. We have noted a high frequency of expression of *ROS* in malignant glioblastomas (Birchmeier et al., *Proc. Natl. Acad. Sci. 84:* 9270 [1987]), and as such, *ROS* may encode a surface antigen useful for diagnosis and possibly therapeutic intervention.

We have continued our study of *ROS* in three veins: First, we have developed polyclonal antibodies to the *ROS* product. These recognize a 270-kD glycoprotein in most cells that express *ROS*. As such, *ROS* would encode one of the largest known membrane receptor/kinases. In collaboration with Tom Jessel at Columbia University, these antibodies have been used to study the expression of *ROS* in developing rat embryos. Our data suggest that *ROS* is normally expressed on radial glial cells in the developing spinal cord.

Second, we have cloned most and perhaps all of the cDNA of the large 8.3-kb *ROS* transcript found in glioblastoma cells. DNA sequencing is not yet complete, but it is apparent that *ROS* encodes a protein that is most similar to the *Drosophila sevenless* product (Hafen et al., *Science 236:* 55 [1987]; Basler and Hafen, *Cell 54:* 299 [1988]; Bowtell et al., *Genes Dev. 2:* 260 [1988]). *sevenless* encodes a protein required for the proper development of fly retinal cells. One interesting possibility is that *ROS* encodes a membrane receptor important in cell-cell recognition.

Third, we have begun to characterize abnormal *ROS* expression in one glioblastoma cell line, U-118 MG. In this cell, the *ROS* locus has fused with a second locus, and a chimeric transcript and protein result. Analysis of the cDNA for this transcript indicates that the loss of the extracellular and transmembrane domains of *ROS* has occurred, leaving the tyrosine kinase domain intact. Just such a rearrangement has been seen in the *MET* oncogene (Dean et al., *Nature 318:* 385 [1985]) and suggests that the rearrangement has activated the oncogenic potential of *ROS*. One other peculiar feature of the rearranged *ROS* in U-118 MG is that the normal *ROS* allele is absent. This and other data suggest that the *ROS* rearrangement has resulted from an intrachromosomal deletion of chromosome 6 and a loss of the normal chromosome 6. We will further characterize the nature of this event.

The *MAS* Oncogene

D. Young, L. Rodgers

The human *MAS* oncogene was discovered by us using the cotransfection and tumorigenicity assay (Young et al., *Cell 45:* 711 [1985]). It encodes a protein with seven transmembrane domains and probably belongs to the family of genes encoding hormone receptors that couple to G proteins. Cells transformed by *MAS* grow to high cell density and form tumors in nude mice but do not appear morphologically transformed and do not grow in suspension in soft agar. Recently, we cloned the rat homolog of *MAS* (Young et al., *Proc. Natl. Acad. Sci. 85:* 5339 [1988]). It encodes a protein very similar to the human protein. In rats, the expression of *MAS* appears to be restricted to the brain, particularly the cerebral cortex and hippocampus, suggesting that *MAS* encodes a receptor for a neurotransmitter (Young et al., *Proc. Natl. Acad. Sci. 85:* 5339 [1988]). Most recently, Hanley and co-workers in England have reported evidence that angiotensin II is a ligand for the *MAS* protein (Jackson et al., *Nature 335:* 437 [1988]). In collaboration with Richard Axel and Tom Jessel at Columbia University, we are attempting to confirm these results (so far unsuccessfully).

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