ONCOGENES AND CANCER, S. A. AARONSON ET AL. (EDS.),
JAPAN SCI. SOC. PRESS, TOKYO/VNU SCI. PRESS, UTRECHT, PP. 253-260, 1987

Exploring the Function of *RAS* Oncogenes by Studying the Yeast *Saccharomyces cerevisiae*

Takashi TODA, Daniel BROEK, Jeffrey FIELD, Tamar MICHAELI, Scott CAMERON, Junichi NIKAWA, Philip SASS, Carmen BIRCHMEIER, Scott POWERS, and Michael WIGLER

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, U.S.A.

Abstract: The *RAS* oncogenes comprise 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*). 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. However, *RAS* proteins do not appear to stimulate adenylate cyclase in vertebrate cells. Our studies indicate that although *RAS* proteins are essential controlling elements of adenylate cyclase in yeast, they have other essential functions in that organims. *RAS* proteins are themselves probably controlled by growth regulatory proteins.

Oncogenes are most concisely defined as minimal genetic elements which can induce the malignant transformation of nontumorigenic cells. One of the great themes to emerge from recent oncogene research is that oncogenes are involved in signal transduction pathways. Several oncogenes have now been discovered to encode growth factor receptors or growth factors themselves (*1–5*). Others, the nuclear oncogenes *fos* and members of the *myc* family, are transcriptionally induced by extracellular signals (*6–8*). Direct connections are suspected between signal pathways and many of the oncogenes which encode the serine/threonine and tyrosine kinases, since other kinases, such as protein kinase C and the cAMP dependent protein kinases, are known to be parts of signal transduction pathways (*9*, *10*).

The *RAS* proteins, like many of the known oncoproteins, are associated with the inner surface of the cell membrane (*11–14*). They are guanine nucleotide binding proteins which have weak GTP hydrolysis activity (*15–18*). Certain oncogenic forms of *RAS* proteins have diminished GTPase activity (*16–18*). Based on these facts, and reasoning by analogy to the "G" proteins, which mediate many hormonal responses, bind GTP, have GTPase activity and are membrane associated (*19*), it seems likely

that the *RAS* proteins are also involved in signal transduction. However, neither the signaling receptors for *RAS* proteins nor the *RAS* effector pathways have been defined in mammalian cells.

Both Yeast and Human RAS Proteins Can Stimulate the Yeast Adenylate Cyclase

In order to understand better *RAS* function, we have turned to a study of *RAS* in the simple eukaryote, the yeast *Saccharomyces cerevisiae*. This is possible because the *RAS* genes have been highly conserved in evolution (*20–23*). The *RAS* genes are particularly amenable to study in yeast because of the excellent genetic tools available for manipulating that organism. There are two yeast *RAS* homologs, *RAS1* and *RAS2*, which form an essential but complementary pair (*24–26*). If properly expressed, the human H-*ras* gene can actually suppress the lethality that otherwise results from loss of both yeast *RAS* genes (*25, 27*). Thus yeast and mammalian *RAS* have conserved biochemical function. Like the mammalian proteins, the yeast *RAS* encode membrane associated guanine nucleotide binding proteins with weak GTPase activity (*28, 29*). The mutant yeast *RAS2*^{val19} protein, like its analogous oncogenic mammalian counterpart, has a reduced GTPase activity and a dominant phenotype. This phenotype is best characterized as an inability to respond properly to signals of nutrient deprivation: failure to G1 arrest, accumulate glycogen, sporulate or become heat shock resistant when starved (*24, 25, 30*).

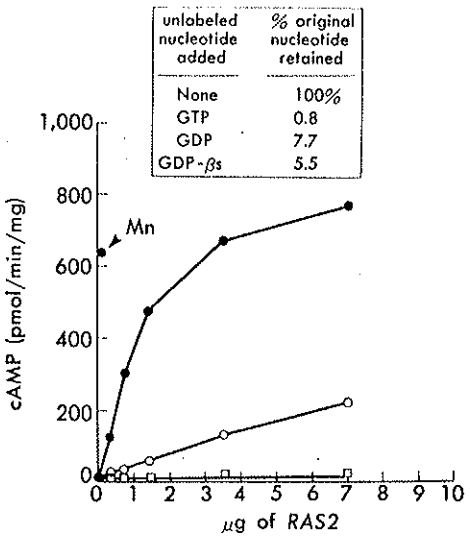

FIG. 1. Response of yeast adenylate cyclase to *RAS2* bound to various guanine nucleotides. To monitor nucleotide exchange, *RAS2* purified from an *Escherichia coli* expression system was first bound to ^{3}H-GDP and then, where indicated, incubated with an excess of unlabeled guanine nucleotide until nitrocellulose filter binding assays indicated that >90% of the label was exchanged (see inset). Next the indicated amounts of *RAS2* bound to GTP (●), GDP (○) or GDP-βS (□) (guanosine-5′-O-(2-thiodiphosphate)) were used in adenylate cyclase assays containing 10 μg of yeast membrane protein prepared from a strain that overexpresses yeast adenylate cyclase. Mg^{2+} was used as the divalent cation, except when indicated by Mn^{2+}. The data is expressed as cAMP produced per min per mg of yeast membrane protein.

TABLE 1. Intracellular cAMP Levels in *ras*⁻ and *cyr1*⁻ Strains

Genotype	cAMP (pmol)/mg protein	Limit of detection (pmol/mg protein)
RAS1 RAS2 CYR1	2.77	0.14
RAS1 RAS2 cyr1⁻	<0.08	0.08
ras1⁻ *ras2*⁻ *CYR1*	0.09	0.03
ras1⁻ *ras2*⁻ *cyr1*⁻	0.07	0.05

ras⁻ and *cyr1*⁻ strains contain an extrachromosomal suppressor plasmid, pYE*TPK1* that carries the *TPK1* gene, a part of 2μ plasmid and the *LEU* 2 gene as a selectable marker. pYE*TPK1* ensures normal growth of these strains.

Early genetic studies indicated a similarity between the phenotype of the *RAS2*^{val19} mutation and mutations in *bcy1*, the gene which encodes the regulatory subunit of the cAMP dependent protein kinase (*31–33*). Biochemical analysis shows that yeast *RAS* proteins, and mammalian proteins as well, are potent stimulators of the yeast adenylate cyclase present in crude membrane preparations (*34*). This stimulation is strongly GTP dependent (see Fig. 1), confirming models of *RAS* function in which intrinsic GTP hydrolysis rates determined the longevity or strength of "signals" transmitted by *RAS* proteins. A second point of these studies is that adenylate cyclase appears to be entirely *RAS* dependent both in the intact cell and in *in vitro* biochemical assays in the presence of magnesium ions (see Fig. 1 and Table 1).

These studies strongly suggest that, in yeast, one effector system for *RAS* is adenylate cyclase. Moreover, mutations in genes of the cAMP effector pathway can suppress the lethality which otherwise results from loss of *RAS* function (*35, 36*). We thus conclude that if yeast *RAS* has a function besides stimulating adenylate cyclase, that function is partially redundant with the functions of cAMP.

A Possible Second Function of Yeast RAS Proteins

It was therefore disturbing when we discovered that the oncogenic human H-*ras*^{val12} protein induces maturation when injected into *Xenopus laevis* oocytes without altering cAMP production (*37*). This observation left the paradox that the mammalian H-*ras* could function in yeast, could stimulate yeast adenylate cyclase there, and yet could function in a vertebrate cell without evidently altering adenylate cyclase.

A potential solution to this paradox has begun to emerge from a more detailed genetic analysis of *RAS* function in *Saccharomyces cerevisiae*. The yeast adenylate cyclase gene, *CYR1*, was cloned (*36*). Gene disruption experiments clearly indicated that *CYR1* encodes the only detectable adenylate cyclase of *Saccharomyces cerevisiae* (see Table 1). We next compared the viability of cells lacking adenylate cyclase activity to cells lacking *RAS* proteins. To do this, we constructed heterozygous diploid yeast strains, and examined the ability of haploid progeny to form colonies. The surprising result (Table 2) was that whereas progeny lacking *RAS1* and *RAS2* were completely inviable, progeny lacking *CYR1* were often capable of forming very slowly growing colonies. This result suggested that the *RAS* proteins in yeast have a critical function in addition to stimulation of adenylate cyclase.

A second and independent line of evidence points to the same conclusion. In the

TABLE 2. Tetrad Dissection of *ras1*$^-$/+ *ras2*$^-$/+ *cyr1*$^-$/+ Diploids

Genotype of haploid progeny			Viable	Non-viable
RAS1	*RAS2*	*CYR1*		
+	+	+	21	0
–	+	+	18	0
+	–	+	23	0
+	+	–	13	7
+	–	–	1	3
–	+	–	5	5
–	–	+	0	7
–	–	–	0	14

The genotypes of non-viable spores were assigned on the basis of the viable spores within the same tetrad, assuming normal Mendelian segregation of genetic loci. *cyr1*$^-$ cells grow very slow and these colonies can barely be seen after a week incubation upon germination.

course of our work, we succeeded in cloning the genes in yeast which encode the cAMP dependent protein kinase catalytic subunits. There are three such genes, named *TPK1*, *TPK2*, and *TPK3*. We examined the ability of a high copy plasmid expressing *TPK1*, pYR*TPK1*, to suppress the phenotypic effects of loss of *RAS* function or loss of adenylate cyclase activity. As expected, cells lacking *CYR1* grew well at both 30°C and 37°C when they contained the pYR*TPK1* plasmid, consistent with the widely held notion that the only essential effects of cAMP are mediated through its stimulation of the cAMP dependent protein kinase. In contrast to this result, cells lacking *RAS1* and *RAS2* but containing pYR*TPK1* could grow well only at 30°C, indicating that overexpression of the cAMP dependent protein kinase catalytic subunit only partially suppresses loss of *RAS* function.

We are led by these results to conclude that *RAS* must have some essential function in yeast besides the stimulation of adenylate cyclase. Since loss of *RAS* function can be suppressed by a dramatic increase in the activity of the cAMP dependent protein kinase, this other essential function must be partially redundant with the cAMP dependent kinase system. It has been suggested that the yeast *RAS* proteins also modulate phospholipid turnover (*38*) although the data for this is not convincing. It is likely that either *RAS* proteins have two distinct primary functions (directly stimulating adenylate cyclase and directly stimulating something else) or that their primary function is as yet unknown, leading indirectly to the stimulation of divergent effector pathways. In both cases, one of the primary functions of *RAS* may have been conserved in evolution.

A Protein That Controls Yeast RAS Proteins

While we have been making progress in understanding the effector functions of *RAS* in yeast, we have also been making progress in defining a protein component in yeast which affects *RAS* function. The *cdc25*^{ts} allele was discovered by Hartwell as a temperature sensitive G1 arrest mutant (*39*, *40*). We found that this temperature sensitive defect is suppressible by high copy plasmids expressing the *RAS2*, or *CYR1* genes (see Table 3); and so we reasoned that *CDC25* might encode a protein in the

TABLE 3. Suppression of *cdc25*^{ts} and *cdc25*$^{-}$ by Multi-copy Plasmids

Gene on multi-copy plasmid	*cdc25*^{ts}	*cdc25*$^{-}$
RAS1	−	−
RAS2	+	−
RAS2^{val}	+	+
CYR1	+	+
TPK1	+	+
TPK2	+	+
TPK3	+	+

All the plasmids used contained a part of 2μ plasmids as a replicator and the *LEU2* gene as a selectable marker. p*RAS1*-2 and p*RAS2*-1 (*22*) were used for *RAS1* and *RAS2* plasmids. p*RAS2*^{val19} was previously described (*41*). p*CYR1* was used for the adenylate cyclase gene (*36*). Plasmids for the *TPK* genes will be described elsewhere.

TABLE 4. Adenylate Cyclase Activity in Yeast Membranes with Disrupted *CDC25*

Strain	Genotype	Adenylate cyclase activity (pmol cAMP/min/mg)		
		Mn^{2+}	Mg^{2+}	Mg^{2+} +GPP(NH)p
Exp. I				
SP1	Wild type	49	2.4	10.0
TT1A-4	*cdc25*$^{-}$ p*CDC25*	56	4.1	10.4
TT1A-3	*cdc25*$^{-}$ p*TPK1*	29	0.1	5.6
Exp. II				
SP1	Wild type	51	3.1	11.3
TT1A-4	*cdc25*$^{-}$ p*CDC25*	40	3.3	9.6
TT1A-3	*cdc25*$^{-}$ p*TPK1*	37	0.3	9.5
Exp. III				
SP1	Wild type	58	4.0	10.5
TT1A-4	*cdc25*$^{-}$ p*CDC25*	42	4.4	9.7
TT1A-3	*cdc25*$^{-}$ p*TPK1*	39	0.2	9.4
Exp. IV				
SP1	Wild type	68	2.1	8.0
TK161-R2V	*RAS2*^{val19}	79	11.7	12.6
TT1A-5	*cdc25*$^{-}$ p*RAS2*^{val19}	71	4.0	8.4

Yeast membrane fractions were prepared from the indicated strains and assayed either in the presence of 2.5 mM $MnCl_2$ or 2.5 mM $MgCl_2$ or 2.5 mM $MgCl_2$, and 10 mM Gpp(NH)p as described (*34*). Gpp(NH)p is a nonhydrolysable analog of GTP. The notation, p*XXX*, indicates that the host strain carries an extrachromosomal plasmid expressing the full length *XXX* gene.

RAS/adenylate cyclase pathway. We therefore cloned *CDC25* by complementation screening. This resulted in the isolation of the *CDC25* gene (*41*), as well as the *TPK1* gene, and enabled us to do a more careful genetic and biochemical analysis of *CDC25* function.

Gene disruptions of *CDC25* proved lethal, but lethality could be suppressed by the high copy plasmids indicated in Table 3. These results differed from results with *cdc25*^{ts} only in that a high copy plasmid expressing *RAS2* does not suppress *cdc25*$^{-}$ whereas a high copy plasmid expressing *RAS*^{val19} does. Thus, cells containing *RAS*^{val19} escape control of *CDC25*, a suggestion that *CDC25* controls normal *RAS* function.

Further evidence for this comes from analysis of adenylate cyclase activity in crude membranes derived from various strains. These results (Table 4) indicate that in *cdc25*$^-$ *RAS2* strains, basal levels of adenylate cyclase activity are drastically reduced when assayed in magnesium, but not when assayed in the presence of manganese ions. This suggests that basal levels of adenylate cyclase are reduced in *cdc25*$^-$ strains. The amount of *RAS* is not reduced, as indicated by the full responsiveness of adenylate cyclase to GTP in the presence of magnesium ions. However, adenylate cyclase activity displays normal basal levels in *cdc25*$^-$ *RAS2*^{val19} strains. We interpret this data to indicate that *CDC25* controls basal *RAS* function in some manner, but the mutant *RAS*^{val19} escapes this control.

CONCLUSIONS

In summary, many unanswered questions about *RAS* remain. We still do not know the precise effector function of *RAS*, nor the chain of command which directs its activity. However we have established several facts about yeast *RAS*. *RAS* regulates events in G1, and is involved in signal transduction (nutritional response). *RAS* controls more than one effector pathway. At least one of these pathways appear to be controlled absolutely in a GTP dependent manner. Ultimately, the *RAS* induced signal passes to a cytoplasmic serine/threonine kinase. Finally, *RAS* in turn appears to be regulated by at least one other protein. We expect these conclusions will be largely valid in mammalian cells.

ACKNOWLEDGMENTS

We are appreciative to P. Bird for preparation of this manuscript. This work was supported by grants from National Institute of Health, the American Business Foundation for Cancer Research, American Cancer Society and Pfizer Biomedical Research Award. M. W. is an American Cancer Society Research Professor.

REFERENCES

1. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J., and Waterfield, M. D. Close similarity of epidermal growth factor receptor and v-*erb*-B oncogene protein sequences. Nature, *307*: 521–527, 1984.
2. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E.L.V., Whittle, N., Waterfield, M. D., and Seeburg, P. H. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature, *309*: 418–425, 1984.
3. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T., and Stanley, E. R. The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell, *41*: 665–676, 1985.
4. Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., DeVare, S. G., Robbins, K. C., Aaronson, S. A., and Antoniades, H. N. Simian sarcoma virus *onc* gene, v-*sis* is derived from the gene (or genes) encoding a platelet-derived growth factor. Science, *221*: 275–276, 1983.
5. Waterfield, M. D., Scrace, G. J., Whittle, N., Stroobant, P., Johnson, A., Wasteson, A., Westermark, B., Heldin, C.-H., Huang, J. S., and Deuel, T. F. Platelet-derived

growth factor is structurally related to the putative transforming protein p28^{sis} of simian sarcoma virus. Nature, *304*: 35–39, 1983.
6. Greenberg, M. E. and Ziff, E. B. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature, *311*: 433–438, 1984.
7. Kruijer, W., Cooper, J. A., Hunter, T., and Verma, I. M. Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. Nature, *312*: 711–716, 1984.
8. Cochran, B. H., Zullo, J., Verma, I. M., and Stiles, C. D. Expression of the c-*fos* gene and of an *fos*-related gene is stimulated by platelet-derived growth factor. Science, *226*: 1080–1082, 1984.
9. Nishizuka, Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature, *308*: 693–698, 1984.
10. Krebs, E. G. and Beavo, J. A. Phosphorylation-dephosphorylation of enzymes. Annu. Rev. Biochem., *48*: 923–959, 1979.
11. Willingham, M. C., Pastan, I., Shih, T. Y., and Scolnick, E. M. Localization of the *src* gene product of the Harvey strain of MSV to plasma membrane of transformed cells by electron microscopic immunocytochemistry. Cell, *19*: 1005–1014, 1980.
12. Shih, T. Y., Weeks, M. O., Gruss, P., Dhar, R., Oroszland, S., and Scolnick, E. M. Identification of a precursor in the biosynthesis of the p21 transforming protein of Harvey murine sarcoma virus. J. Virol., *42*: 253–261, 1982.
13. Clark, S. G., McGrath, J. P., and Levinson, A. P. Expression of normal and activated human Ha-*ras* cDNAs in *Saccharomyces cerevisiae*. Mol. Cell. Biol., *5*: 2746–2752, 1985.
14. Fujiyama, A. and Tamanoi, F. Processing and fatty acid acylation of RAS1 and RAS2 proteins in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A., *83*: 1266–1270, 1986.
15. Scolnick, E. M., Papageorge, A. G., and Shih, T. Y. Guanine nucleotide-binding activity as an assay for *src* protein of rat-derived murine sarcoma viruses. Proc. Natl. Acad. Sci. U.S.A., *76*: 5355–5359, 1979.
16. Gibbs, J. B., Sigal, I. S., Poe, M., and Scolnick, E. M. Intrinsic GTPase activity distinguishes normal and oncogenic *ras* p21 molecules. Proc. Natl. Acad. Sci. U.S.A., *81*: 5704–5708, 1984.
17. McGrath, J. P., Capon, D. J., Goeddel, D. V., and Levinson, A. D. Comparative biochemical properties of normal and activated human *ras* p21 protein. Nature, *310*: 644–649, 1984.
18. Sweet, R., Yokoyama, S., Kamata, T., Feramisco, J., Rosenberg, M., and Gross, M. The product of *ras* is a GTPase and the T24 oncogenic mutant is deficient in this activity. Nature, *311*: 273–275, 1984.
19. Gilman, A. G proteins and dual control of adenylate cyclase. Cell, *36*: 577–579, 1984.
20. Shilo, B-Z. and Weinberg, R. A. DNA sequences homologous to vertebrate oncogenes are conserved in *Drosophia melanogaster*. Proc. Natl. Acad. Sci. U.S.A., *78*: 6789–6792, 1981.
21. DeFeo-Jones, D., Scolnick E. M., Zoller, R., and Dhar, R. *ras*-related gene sequences identified and isolated from *Saccharomyces cerevisiae*. Nature, *306*: 707–709, 1983.
22. Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Strathern, J., Broach, J., and Wigler, M. Genes in *S. cerevisiae* encoding proteins with domains homologous to the mammalian *ras* proteins. Cell, *36*: 607–612, 1984.
23. Reymond, C. D., Gomer, R. H., Mehdy, M. C., and Firtel, R. A. Developmental regulation of a dicytostelium gene encoding a protein homologous to mammalian *ras* protein. Cell, *39*: 141–148, 1984.
24. Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J., Broach, J., and Wigler, M. Genetic analysis of yeast RAS1 and RAS2 genes. Cell, *37*: 437–445, 1984.

25. Kataoka, T., Powers, S., Cameron, S., Fasano, O., Goldfarb, M., Broach, J., and Wigler, M. Functional homology of mammalian and yeast *RAS* genes. Cell, *40*: 19–26, 1985.
26. Tatchell, K., Chaleff, D., DeFeo-Jones, D., and Scolnick E. Requirement of either of a pair of *ras*-related genes of *Saccharomyces cerevisiae* for spore viability. Nature, *309*: 523–527, 1984.
27. DeFeo-Jones, D., Tatchell, K., Robinson, L. C., Sigal, I. S., Vass, W. C., Lowy, D. R., and Scolnick E. M. Mammalian and yeast *ras* gene products: biological function in their heterologous system. Science, *228*: 179–184, 1985.
28. Tamanoi, F., Walsh, M., Kataoka, T., and Wigler, M. A product of yeast *RAS*2 gene is a guanine nucleotide binding protein. Proc. Natl. Acad. Sci. U.S.A., *81*: 6924–6929, 1984.
29. Temeles, G. L., Gibbs, J. B., D'Alonzo, J. S., Sigal, I. S., and Scolnick, E. M. Yeast and mammalian *ras* proteins have conserved biochemical properties. Nature, *313*: 700–703, 1984.
30. Powers, S., Michaelis, S., Broek, D., Santa Anna-A, S., Field, J., Herskowitz, I., and Wigler, M. *RAM*, a gene of yeast required for a functional modification of *RAS* proteins and for production of mating pheromone *a*-factor. Cell, *47*: 413–422, 1986.
31. Matsumoto, K., Uno, I., Oshima, Y., and Ishikawa, T. Isolation and characterization of yeast mutant deficient in adenylate cyclase and cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. U.S.A., *79*: 2355–2359, 1982.
32. Matsumoto, K., Uno, I., and Ishikawa, T. Control of cell division in *Saccharomyces cerevisiae* mutants defective in adenylate cyclase and cAMP-dependent protein kinase. Exp. Cell. Res., *146*: 151–161, 1983.
33. Toda, T., Cameron, S., Sass, P., Zoller, M., Scott, J. D., McMullen, B., Hurwitz, M., Krebs, E. G., and Wigler, M. Cloning and characterization of *BCY1*, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. Mol. Cell. Biol., 1987 (in press).
34. Broek, D., Samiy, N., Fasano, O., Fujiyama, A., Tamanoi, F., Northup, J., and Wigler, M. Differential activation of yeast adenylate cyclase by wild-type and mutant *RAS*, product. Cell, *41*: 763–769, 1985.
35. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K., and Wigler, M. In yeast, *RAS* proteins are controlling elements of adenylate cyclase. Cell, *40*: 27–36, 1985.
36. Kataoka, T., Broek, D., and Wigler, M. DNA sequence and characterization of the *S. cerevisiae* gene encoding adenylate cyclase. Cell, *43*: 493–505, 1985.
37. Birchmeier, C., Broek, D., and Wigler, M. *RAS* proteins can induce meiosis in Xenopus oocytes. Cell, *43*: 615–621, 1985.
38. Kaibuchi, K., Miyajima, A., Arai, K-I., and Matsumoto, K. Possible involvement of *RAS*-encoded proteins in glucose-induced inositolphospholipid turnover in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A., *83*: 8172–8176, 1986.
39. Hartwell, L. H., Mortiner, R. K., Culotti, J., and Culotti, M. Genetic control of the cell division cycle in yeast: V. genetic analysis of *cdc* mutant. Genetics, *74*: 267–286, 1973.
40. Hartwell, L. H. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev., *38*: 164–198, 1974.
41. Broek, D., Toda, T., Michaeli, T., Levin, L., Birchmeier, C., Zoller, M., Powers, S., and Wigler, M. The *S. cerevisiae CDC25* gene product regulates the RAS/adenylate cyclase pathway. Cell (in press).