Requirement of One Functional *RAS* Gene and Inability of an Oncogenic *ras* Variant To Mediate the Glucose-Induced Cyclic AMP Signal in the Yeast *Saccharomyces cerevisiae*

KAISHUSHA MBONYI, MONIQUE BEULLENS, KATRIEN DETREMERIE, LIEVE GEERTS,
AND JOHAN M. THEVELEIN*

*Laboratorium voor Cellulaire Biochemie, Katholieke Universiteit te Leuven, Kardinaal Mercierlaan 92,
B-3030 Leuven-Heverlee, Flanders, Belgium*

Received 11 December 1987/Accepted 18 April 1988

Addition of glucose to *Saccharomyces cerevisiae* cells grown on a nonfermentable carbon source triggers a cyclic AMP (cAMP) signal, which induces a protein phosphorylation cascade. In a yeast strain lacking functional *RAS1* and *RAS2* genes and containing a *bcy* mutation to suppress the lethality of *RAS* deficiency, the cAMP signal was absent. Addition of dinitrophenol, which stimulates *in vivo* cAMP synthesis by lowering intracellular pH, also did not enhance the cAMP level. A *bcy* control strain, with functional *RAS* genes present, showed cAMP responses similar to those of a wild-type strain. In disruption mutants containing either a functional *RAS1* gene or a functional *RAS2* gene, the cAMP signal was not significantly different from the one in wild-type cells, indicating that *RAS* function cannot be a limiting factor for cAMP synthesis during induction of the signal. Compared with wild-type cells, the cAMP signal decreased in intensity with increasing temperature in a *ras2* disruption mutant. When the mutant *RAS2*^{Val-19}, which carries the equivalent of the human H-*ras*^{Val-12} oncogene, was grown under conditions in which *RAS1* expression is repressed, the cAMP signal was absent. The oncogene product is known to be deficient in GTPase activity. However, the amino acid change at position 19 (or 12 in the corresponding human oncogene product) might also have other effects, such as abolishing receptor interaction. Such an additional effect probably provides a better explanation for the lack of signal transmission than the impaired GTPase activity. When the *RAS2*^{Val-19} mutant was grown under conditions in which *RAS1* is expressed, the cAMP signal was present but significantly delayed compared with the signal in wild-type cells. This indicates that oncogenic *RAS* proteins inhibit normal functioning of wild-type *RAS* proteins *in vivo* and also that in spite of the presence of the *RAS2*^{Val-19} oncogene, adenylyl cyclase is not maximally stimulated *in vivo*. Expression of only the *RAS2*^{Val-19} gene product also prevented most of the stimulation of cAMP synthesis by dinitrophenol, indicating that lowered intracellular pH does not act directly on adenylyl cyclase but on a step earlier in the activation pathway of the enzyme. The results obtained with the control *bcy* strain, the *RAS2*^{Val-19} strain under conditions in which *RAS1* is expressed, and with dinitrophenol show that the inability of the oncogene product to mediate the cAMP signal is not due to feedback inhibition by the high protein kinase activity in strains containing the *RAS2*^{Val-19} oncogene. Hence, the present results show that the *RAS* proteins in *S. cerevisiae* are involved in the transmission of the glucose-induced cAMP signal and that the oncogenic *RAS* protein is unable to act as a signal transducer. The *RAS* proteins in *S. cerevisiae* apparently act similarly to the *G_s* proteins of mammalian adenylyl cyclase, but instead of being involved in hormone signal transmission, they function in a nutrient-induced signal transmission pathway.

Addition of glucose or related fermentable sugars to *Saccharomyces cerevisiae* cells grown on nonfermentable carbon sources (derepressed cells), stationary-phase cells, or ascospores induces a cyclic AMP (cAMP) signal which triggers a protein phosphorylation cascade. This cascade is responsible for rapid depletion of the storage sugar trehalose, for inhibition of gluconeogenesis, and for stimulation of glycolysis (for a review, see reference 39a). The mechanism by which glucose triggers the cAMP signal is not known. At first, rather unspecific mechanisms such as transient plasma membrane depolarization (23, 31) and transient intracellular acidification (10, 36, 46) were proposed. However, these hypotheses were shown to be wrong, and other rather unspecific mechanisms, such as increased energy supply, were also excluded (17, 41, 42). On the other hand, it was confirmed that drastic lowering of the intracellular pH in yeast cells strongly stimulates *in vivo* cAMP synthesis (41). This effect has been attributed up to now to the difference in

pH optima of adenylyl cyclase ($\pm$ pH 6) and phosphodiesterase ($\pm$ pH 8) (28, 29). In this paper, evidence is presented that lowered intracellular pH does not act directly on adenylyl cyclase but rather on the *RAS* proteins or on other controlling elements situated earlier in the pathway for activation of adenylyl cyclase.

Recent studies with kinase mutants of *S. cerevisiae* showed that for induction of the cAMP signal by glucose, at least one of the three kinases, hexokinase 1, hexokinase 2, or glucokinase, has to be present. For induction of the signal by fructose, at least one of the two hexokinases has to be present (1). Whether the requirement for the corresponding kinase reflects a requirement for phosphorylation of the sugar or for the presence of the high-affinity sugar transport system (2) was not clear at that time. Recent results with a mutant disrupted in the *SNF3* gene, which codes for the high-affinity glucose carrier (4, 33), clearly show that this carrier is not needed for induction of the cAMP signal by glucose or fructose (Mbonyi and Thevelein, submitted for publication).

* Corresponding author.

In mammalian cells, proteins of the G class, called G_s , are involved in the induction of cAMP signals by hormones. These proteins bind GTP, and in this form they activate adenylyl cyclase. Their own GTPase activity converts them back to the inactive GDP-bound form (22). Other G class proteins in mammalian cells are the RAS proteins, which are also localized in the plasma membrane and of which oncogenic variants exist which have greatly reduced GTPase activity (20, 32, 38). Recently, the yeast *S. cerevisiae* has been shown to contain two genes, *RAS1* and *RAS2*, which are both structurally and functionally homologous to the mammalian RAS gene (13, 14, 16, 25, 35). In addition, it was shown that the yeast RAS proteins are controlling elements of adenylyl cyclase and that the presence of an equivalent of the human *H-ras*^{Val-12} oncogene, *RAS2*^{Val-19}, results in overstimulation of adenylyl cyclase in vitro and higher cAMP levels in vivo (6, 26, 43). Data consistent with these observations were also published for *RAS1* oncogene equivalents (30). However, in vivo studies of the relationship between cAMP and RAS in yeast cells have been very limited up to now. Only two papers have been published in which in vivo cAMP levels in *ras* mutants were reported (34, 43).

Although there has been speculation that the RAS proteins of *S. cerevisiae* are in some way involved in sensing the nutritional status of the medium (37, 39), no direct evidence in this respect has been reported. In the present paper it is shown that the RAS proteins are involved in transmission of the glucose-induced cAMP signal in yeast cells grown on nonfermentable carbon sources. The data therefore indicate that the mechanism of this nutrient-induced cAMP signal shows analogy to the mechanism of the hormone-induced cAMP signal in mammalian cells and that the RAS proteins in yeast cells fulfill a function similar to that of the signal-transducing G_s proteins in mammalian cells.

MATERIALS AND METHODS

Yeast strains. The following yeast strains were provided by M. Wigler (Cold Spring Harbor Laboratory): SP1 (wild type) (*MATa leu2 his3 trp1 ade8 can1 RAS1 RAS2*), TK161-R2V (same as SP1 but *RAS2*^{Val-19} *RAS1*), ST-1 (same as SP1 but *ras1::URA3 RAS2*), KP-2 (same as SP1 but *ras2::URA3 RAS1*), T16-11A (*MATa leu2 ura3 trp1 his3 bcy1 RAS1 RAS2*), and T23-13B (same as T16-11A but *ras1::HIS3 ras2::URA3*).

Culture conditions. The cells were grown to a density of about 8 mg (wet weight) ml⁻¹ in a gyratory incubator (200 rpm) at 30°C. The culture medium contained 3% glycerol, 2% peptone, and 1% yeast extract. The culture medium in which expression of *RAS1* was repressed contained 3% glycerol and 1% yeast extract and was adjusted to pH 7.6 with KOH (5, 19). The cultures were cooled in ice before harvesting, and the cells were washed twice with ice-cold 25 mM MES buffer (morpholineethanesulfonic acid, pH 6).

Incubation conditions. The cells were incubated at a density of 30 mg (wet weight) ml⁻¹ in a reciprocating water bath shaker at a temperature of 25, 32, or 37°C as indicated in the figure legends. Incubation was carried out in 25 mM MES buffer, pH 6, for 10 min before addition of 100 mM glucose or 2 mM 2,4-dinitrophenol (DNP).

cAMP determination. Determination of cAMP levels was performed as described previously (41). All experiments were repeated at least three times with consistent results. Representative results are shown.

FIG. 1. Glucose-induced cAMP signal at 25°C in derepressed cells of wild-type (●), *ras1 RAS2* (○), and *RAS1 ras2* (▲) strains.

RESULTS

Addition of 100 mM glucose to wild-type yeast cells grown on nonfermentable carbon sources triggered a rapid transient increase in the cAMP level (Fig. 1). Addition of the same amount of glucose to cells containing either a disrupted *RAS1* or *RAS2* gene triggered a cAMP signal of about the same intensity (Fig. 1). Addition of 2 mM DNP caused a rapid and drastic increase in the cAMP level in all three strains (results not shown). Yeast strains containing both disrupted *RAS1* and *RAS2* genes have very low cAMP levels and can only grow when they contain an additional mutation (*bcy1*) which renders cAMP-dependent protein kinase cAMP independent (43). Plasma membranes isolated from *ras1 ras2 bcy1* strains, however, still show adenylyl cyclase activity, but only when measured with Mn^{2+} , not with Mg^{2+} and GTP (43). Addition of glucose or DNP to cells of a *ras1 ras2 bcy1* strain did not increase the cAMP level, indicating that adenylyl cyclase activity in vivo is tightly linked to RAS protein activity (Fig. 2). Even conditions which drastically stimulated cAMP synthesis in wild-type cells, such as addition of DNP, had no effect in the absence of functional RAS proteins. Control experiments with a *RAS1 RAS2 bcy1* strain showed that the *bcy1* mutation was not responsible for the absence of a cAMP increase (Fig. 2).

Although these results show that the RAS proteins are needed for the glucose-induced cAMP signal, they did not allow us to conclude whether the RAS proteins acted as transducers of the signal or whether they were simply necessary for adenylyl cyclase activity. Different approaches were used to distinguish between these two possibilities. The glucose-induced cAMP signal was also measured at different temperatures. In the wild-type cells, higher cAMP signals were observed at higher temperatures (Fig. 3). (This is also true for other strains [1]). In a *ras2* disruption mutant, however, the cAMP signal decreased in intensity with increasing temperature (Fig. 3). This result shows that RAS deficiency affects induction of the cAMP signal. In this case too, however, the theoretical possibility remains that the ability of *RAS1* to sustain adenylyl cyclase activity decreases at higher temperatures.

FIG. 2. Glucose-induced cAMP signal (a) and DNP-induced cAMP increase (b) at 32°C in derepressed cells of *ras1 ras2 bcy1* (●) and *bcy1* (○) strains.

When the glucose-induced cAMP signal was measured in the *RAS2^{Val-19}* mutant, the cAMP increase was clearly delayed and in most cases was also significantly smaller than the cAMP increase in wild-type cells (Fig. 4). In these cells, however, the *RAS1* gene is still intact, and the remaining cAMP signal could be due to *RAS1* protein function. This strain therefore was grown under conditions in which *RAS1* expression is largely repressed (5, 19). In these cells, the glucose-induced cAMP signal was barely detectable (Fig. 5a). The very small remaining increase, which also appeared to be shifted as in Fig. 4, is probably due to remaining *RAS1* protein. A similar result was obtained for the DNP-induced cAMP increase: in a *RAS2^{Val-19}* strain, in which *RAS1* expression is repressed, the DNP effect was much less than in cells containing normal *RAS* proteins (Fig. 5b), including cells of a *bcy* strain (Fig. 2). The remaining DNP-induced effect was probably due for the most part to remaining expression of *RAS1* proteins, although an additional direct

pH effect on adenylyl cyclase cannot be excluded. The control experiments with a *ras1* disruption mutant and with wild-type cells in which *RAS1* expression was repressed clearly showed the difference between the functioning of wild-type *RAS2* protein and its oncogenic variant, *RAS2^{Val-19}* (Fig. 5).

Basal cAMP levels in derepressed cells of the *RAS2^{Val-19}* mutant, incubated in MES buffer under aerobic conditions, were about two- to threefold higher than in wild-type cells incubated under the same conditions (Fig. 4 and 5). This difference is somewhat smaller than has been published before for cells of the *RAS2^{Val-19}* mutant growing in a glucose-containing rich medium (43). Recent results from the same group also showed a difference of only a factor of two between the cAMP level in wild-type cells and in cells containing the *RAS2^{Val-19}* oncogene (34).

DISCUSSION

The results presented in this paper show that a specific regulatory mechanism is involved in induction of the cAMP signal by fermentable carbon sources in derepressed yeast cells. The mechanism appears to be analogous to the mechanism of the hormone-induced G protein-mediated cAMP signals in mammalian cells. The nutrient-induced cAMP signal in *S. cerevisiae* is mediated by the *RAS* proteins, which belong to the same class of GTP-binding proteins. The strongest evidence for involvement of the *RAS* proteins was the absence of a glucose-induced cAMP signal in yeast cells containing only the *RAS2^{Val-19}* protein (Fig. 5a). In these cells, however, the basal cAMP level was higher than in wild-type cells (Fig. 5a), and evidence has been presented that elevated protein kinase activity in yeast causes feedback inhibition of cAMP synthesis (34). Several results, however, show that the absence of cAMP signal induction by the *RAS2^{Val-19}* protein is not due to feedback inhibition of cAMP synthesis. (i) The presence of the *RAS2^{Val-19}* mutation in the presence or absence of *RAS1* expression led in both cases to equally increased basal cAMP levels (Fig. 4, Fig. 5a), thus most likely resulting in equally stimulated feedback inhibition of cAMP synthesis. However, when *RAS1* was expressed, a clear (albeit delayed) cAMP signal was observed, which shows that even under conditions of increased feedback inhibition, a cAMP signal can be observed. (ii) Increased feedback inhibition must also be present in *bcy* strains, at least as much as in *RAS2^{Val-19}* strains. In spite of this, a glucose-induced cAMP signal was observed in *bcy* strains (Fig. 2a). (iii) Addition of DNP (or other agents that lower intracellular pH) to yeast cells causes a large increase in the cAMP level (10, 31, 36, 40, 41, 45). The resulting cAMP level is between 10 and 20 times higher than the cAMP level in *RAS2^{Val-19}* strains. Therefore, lowering of the intracellular pH must in some way inactivate or at least greatly reduce feedback inhibition of cAMP synthesis in *S. cerevisiae*. Nevertheless, in the absence of feedback inhibition of cAMP synthesis, we still observed a strongly reduced stimulation by DNP of cAMP synthesis in the *RAS2^{Val-19}* strain under conditions in which *RAS1* was not expressed (Fig. 5b). This clearly shows that even under conditions with no feedback inhibition, the *RAS2^{Val-19}* protein is inefficient in mediating stimulation of adenylyl cyclase. In addition to these arguments, there is also the preliminary evidence presented by Nikawa et al. (34) that the *RAS2^{Val-19}* protein is not subject to control by feedback inhibition. If this could be confirmed, it would render the present problem of possible interference by feedback inhibition superfluous. The results presented in this paper, however, clearly allow us to con-

FIG. 3. Glucose-induced cAMP signal in derepressed cells of the wild-type (●) and *RAS1 ras2* (○) strains at (a) 25°C, (b) 32°C, and (c) 37°C.

clude that the *RAS2*^{Val-19} oncogene product is deficient in the ability to transmit signals to adenylyl cyclase.

The small remaining DNP-induced stimulation of cAMP synthesis in the *RAS2*^{Val-19} strain (Fig. 5b) is probably for the most part due to the small amount of *RAS1* expression that is undoubtedly still present in these cells, although an additional direct pH effect on adenylyl cyclase cannot be excluded. The result obtained with the *RAS2*^{Val-19} strain (Fig. 5b) now makes it very clear that the old pH hypothesis of adenylyl cyclase regulation, which was based on the difference between the pH optima of adenylyl cyclase (pH 6) and

phosphodiesterase (pH 8) (8, 28), can explain the in vivo changes in the cAMP level only to a very limited extent. Most of the artificial stimulation of in vivo cAMP synthesis by lowered intracellular pH must be attributed to activation of a protein somewhere in the control pathway of adenylyl cyclase: the RAS proteins (Fig. 5b) or even earlier, e.g., at CDC25 (L. Van Aelst, unpublished results). As opposed to the glucose-induced cAMP signal, however, sugar kinase activity is not needed for the DNP-induced cAMP increase in *S. cerevisiae* (1).

The RAS proteins in *S. cerevisiae* activate adenylyl cyclase in the GTP-bound form, and their own GTPase activity converts the bound GTP into GDP, which renders the protein inactive (18). It is tempting to speculate that the glucose-induced cAMP signal in wild-type cells is triggered by a sudden stimulation of GDP-GTP exchange on the RAS proteins (39a). Since the *RAS2*^{Val-19} protein has greatly reduced GTPase activity, it cannot display GTP-GDP exchange and is therefore apparently unable to mediate cAMP signals. An alternative explanation is that the amino acid change at position 19 of the RAS protein not only reduces GTPase activity but also has other effects on the functioning of the protein, such as abolishing receptor interaction. The latter probably provides a better explanation for the lack of signal transmission than the impaired GTPase activity. In general, impaired receptor interaction might be more important for the malfunctioning of *ras* oncogene products than reduced GTPase activity. Similar conclusions have been obtained recently for the mammalian N-*ras*^{Val-12} and N-*ras*^{Asp-12} oncogene products, which differ greatly in reduction of their GTPase activity but possess equal transforming potency (44). Lack of correlation between GTPase activity and transforming potency was also reported in earlier publications (11, 15, 27).

In general, G proteins are only active in the GTP-bound state (21, 22). Why the peak value of the cAMP signals is much higher than the basal cAMP level in the *RAS2*^{Val-19} mutant is therefore unclear. The answer to this problem might be related to the reason why the cAMP signal is transient. A possible explanation of both effects is feedback inhibition of cAMP synthesis by high protein kinase activity (34). This could explain why the cAMP level can still increase further in strains with an adenylyl cyclase that is "continuously stimulated" by the *RAS2*^{Val-19} protein. It also

FIG. 4. Glucose-induced cAMP signal at 25°C in derepressed cells of wild-type (●) and *RAS1 RAS2*^{Val-19} (○) strains. The cells were grown under conditions in which both *RAS1* and *RAS2* were expressed.

FIG. 5. Glucose-induced cAMP signal (a) and DNP-induced cAMP increase (b) at 25°C in derepressed cells of wild-type (●), *ras1* RAS2 (○), and *RAS1* RAS2^{Val-19} (▲) strains. The cells were grown under conditions in which only *RAS2* was expressed.

offers an explanation for the transient nature of the glucose-induced cAMP signal: the signal shuts itself off by inducing high protein kinase activity. A displaced cAMP signal (Fig. 4) therefore lasts about as long as a normal signal. The delay observed in the induction of the cAMP signal in the *RAS2*^{Val-19} strain under conditions in which *RAS1* was expressed (Fig. 4) cannot be due to the high protein kinase activity, because otherwise it should also have been observed in a *bcy* strain, and this was not the case (Fig. 2). Hence, we can safely conclude from this observation that oncogenic RAS proteins inhibit signal transmission by normal RAS proteins. This result provides an explanation for the observation by Kaibuchi et al. (24) that both disruption of *RAS1* and introduction of the *RAS2*^{Val-19} mutation stimulated glucose-induced inositolphospholipid turnover in *S. cerevisiae*. If the RAS proteins are inhibitors of glucose-induced inositolphospholipid turnover, the oncogenic *RAS2*^{Val-19} protein should normally overinhibit this process. However, if the oncogenic RAS proteins can inactivate the

functioning of normal RAS proteins, *RAS2*^{Val-19} would suppress the inhibition by *RAS1*, and therefore its presence would result in stimulation.

A possible candidate for triggering GDP-GTP exchange on the RAS proteins is the CDC25 protein, which has been suggested to be a specific GDP-GTP exchange stimulator (7, 9, 12, 37). Recent results from our laboratory have shown that *cdc25* mutants are also deficient in the glucose-induced cAMP signal (L. Van Aelst, unpublished results). Since disruption of *RAS1* or *RAS2* did not diminish the intensity of the cAMP signal (Fig. 1), RAS function cannot be the limiting factor for induction of the signal.

Up to now, little was known about the regulation of the RAS protein in vivo. Our in vivo measurements of cAMP levels confirm the previous suggestion that only the Mg²⁺ and GTP-dependent adenylyl cyclase activity is relevant for in vivo cAMP synthesis. Hence, adenylyl cyclase activity in *S. cerevisiae* appears to be obligatorily coupled to G protein function. This is also the case in mammalian cells (21). The RAS proteins in yeast cells apparently act like the G_s proteins of mammalian adenylyl cyclase, but instead of being involved in hormone signal transmission, they function in a nutrient-induced signal transmission pathway. For unicellular organisms, like *S. cerevisiae*, nutrients are likely to be the prime regulators of cell proliferation, as opposed to multicellular organisms, in which hormones and specific growth factors are more likely to exert prime control over cellular proliferation. Recent research has shown that in fungi simple nutrient molecules, such as carbon and nitrogen sources, can act as regulatory molecules and can directly trigger cellular regulatory pathways, such as protein phosphorylation, independent of their function as a nutrient molecule (for a recent review, see reference 39a). The results presented in this paper show that the mechanisms they use for this purpose are remarkably analogous to those used in hormone-induced regulatory pathways in higher organisms.

The conclusions made in this paper are only valid when the observed effects are directly due to the absence or modification of the RAS proteins. Another possibility is that absence or modification of the RAS proteins affects expression of proteins which also intervene in induction of the cAMP signal and therefore indirectly affect the presence of the signal. This possibility, however, appears to be only theoretical because of the well-documented in vitro effects of the RAS proteins on adenylyl cyclase activity and because these in vitro results fit very well with the results obtained in vivo.

Previous results from this laboratory have shown that the primary trigger of the glucose-induced cAMP signal is situated somewhere at the level of transport and/or phosphorylation of the sugar (1). Present research focuses on the mechanisms by which initial glucose metabolism activates the CDC25-RAS-adenylyl cyclase pathway, which is responsible for induction of the cAMP signal.

ACKNOWLEDGMENTS

We thank M. Wigler (Cold Spring Harbor Laboratory) for provision of the *ras* mutants.

This work was supported by grants from the Belgian National Fund for Scientific Research (FGWO), the National Bank of Belgium, and the Research Fund of the Katholieke Universiteit te Leuven. Kaishusha Mbonyi is on leave from the University of Kisangani (Zaire) with a fellowship of the Belgian Office for Cooperation with Developing Countries (ABOS).

LITERATURE CITED

1. Beullens, M., K. Mbonyi, L. Geerts, D. Gladines, K. Detremmerie, A. W. H. Jans, and J. M. Thevelein. 1988. Studies on the mechanism of the glucose-induced cAMP-signal in glycolysis and glucose repression-mutants of the yeast *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **172**:227-231.
2. Bisson, L. F., and D. G. Fraenkel. 1983. Involvement of kinases in glucose and fructose uptake by *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **80**:1730-1734.
3. Bisson, L. F., and D. G. Fraenkel. 1984. Expression of kinase-dependent glucose uptake in *Saccharomyces cerevisiae*. *J. Bacteriol.* **159**:1013-1017.
4. Bisson, L. F., L. Neigeborn, M. Carlson, and D. G. Fraenkel. 1987. The *SNF3* gene is required for high-affinity glucose transport in *Saccharomyces cerevisiae*. *J. Bacteriol.* **169**:1656-1662.
5. Brevario, D., A. Hinnebusch, J. Cannon, K. Tatchell, and R. Dhar. 1986. Carbon source regulation of *RAS1* expression in *Saccharomyces cerevisiae* and the phenotypes of *ras2⁻* cells. *Proc. Natl. Acad. Sci. USA* **83**:4152-4156.
6. Broek, D., N. Samiy, O. Fasano, A. Fujiyama, F. Tamanoi, J. Northup, and M. Wigler. 1985. Differential activation of yeast adenylate cyclase by wild-type and mutant *RAS* proteins. *Cell* **41**:763-769.
7. Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zoller, S. Powers, and M. Wigler. 1987. The *Saccharomyces cerevisiae CDC25* gene product regulates the *RAS*/adenylate cyclase pathway. *Cell* **48**:789-799.
8. Busa, W. B., and R. Nuccitelli. 1984. Metabolic regulation via intracellular pH. *Am. J. Physiol.* **246**:R409-R438.
9. Camonis, J. H., M. Kalékine, B. Gondré, H. Garreau, E. Boy-Marcotte, and M. Jacquet. 1986. Characterization, cloning and sequence analysis of the *CDC25* gene which controls the cyclic AMP level of *Saccharomyces cerevisiae*. *EMBO J.* **5**:375-380.
10. Caspani, G., P. Tortora, G. M. Hanozet, and A. Guerriero. 1985. Glucose-stimulated cAMP increase may be mediated by intracellular acidification in *Saccharomyces cerevisiae*. *FEBS Lett.* **186**:75-79.
11. Colby, W. W., J. S. Hayflick, S. G. Clark, and A. D. Levinson. 1986. Biochemical characterization of polypeptides encoded by mutated human *Ha-ras1* genes. *Mol. Cell. Biol.* **6**:730-734.
12. Daniel, J., J. M. Becker, E. Enari, and A. Levitzki. 1987. The activation of adenylate cyclase by guanyl nucleotides in *Saccharomyces cerevisiae* is controlled by the *CDC25* start gene product. *Mol. Cell. Biol.* **7**:3857-3861.
13. Defeo-Jones, D., E. M. Scolnick, R. Koller, and R. Dhar. 1983. *ras*-related gene sequences identified and isolated from *Saccharomyces cerevisiae*. *Nature (London)* **306**:707-709.
14. Defeo-Jones, D., K. Tatchell, L. C. Robinson, I. S. Sigal, W. C. Vass, D. R. Lowy, and E. M. Scolnick. 1985. Mammalian and yeast *ras* gene products: biological function in their heterologous systems. *Science* **228**:179-184.
15. Der, C. J., T. Finkel, and G. M. Cooper. 1986. Biological and biochemical properties of human *ras^H* genes mutated at codon 61. *Cell* **44**:167-176.
16. Dhar, R., A. Nieto, R. Koller, D. Defeo-Jones, and E. Scolnick. 1984. Nucleotide sequence of two *ras^H* related-genes isolated from the yeast *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **12**:3611-3618.
17. Eraso, P., M. J. Mazon, and J. M. Gancedo. 1987. Internal acidification and cAMP increase are not correlated in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **165**:671-674.
18. Field, J., D. Broek, T. Kataoka, and M. Wigler. 1987. Guanine nucleotide activation of, and competition between, *RAS* proteins from *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7**:2128-2133.
19. Fraenkel, D. G. 1985. On *ras* gene function in yeast. *Proc. Natl. Acad. Sci. USA* **82**:4740-4744.
20. Gibbs, J., I. Sigal, M. Poe, and E. Scolnick. 1984. Intrinsic GTPase activity distinguishes normal and oncogenic *ras* p21 molecules. *Proc. Natl. Acad. Sci. USA* **81**:5704-5707.
21. Gilman, A. G. 1984. G proteins and dual control of adenylate cyclase. *Cell* **36**:577-579.
22. Gilman, A. G. 1987. G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* **56**:615-649.
23. Holzer, H. 1984. Mechanism and function of reversible phosphorylation of fructose-1,6-bisphosphatase in yeast, p. 143-154. In P. Cohen (ed.), *Molecular aspects of cellular regulation*, vol. 3. Elsevier, Amsterdam.
24. Kaibuchi, K., A. Miyajima, K. Arai, and K. Matsumoto. 1986. Possible involvement of *RAS*-encoded proteins in glucose-induced inositolphospholipid turnover in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **83**:8172-8176.
25. Kataoka, T., S. Powers, S. Cameron, O. Fasano, M. Goldfarb, J. Broach, and M. Wigler. 1985. Functional homology of mammalian and yeast *RAS* genes. *Cell* **40**:19-26.
26. Kataoka, T., S. Powers, C. McGill, O. Fasano, J. Strathern, J. Broach, and M. Wigler. 1984. Genetic analysis of yeast *RAS1* and *RAS2* genes. *Cell* **37**:437-445.
27. Lacal, J. C., S. K. Srivastava, P. S. Anderson, and S. A. Aaronson. 1986. *ras* p21 proteins with high or low GTPase activity can efficiently transform NIH/3T3 cells. *Cell* **44**:609-617.
28. Londesborough, J. 1977. Characterization of an adenosine 3': 5'-cyclic monophosphate phosphodiesterase from baker's yeast. *Biochem. J.* **163**:467-476.
29. Londesborough, J., and T. Nurminen. 1972. A manganese-dependent adenyl cyclase in baker's yeast, *Saccharomyces cerevisiae*. *Acta Chem. Scand.* **26**:3396-3398.
30. Marshall, M. S., J. B. Gibbs, E. M. Scolnick, and I. S. Sigal. 1987. Regulatory function of the *Saccharomyces cerevisiae RAS* C-terminus. *Mol. Cell. Biol.* **7**:2309-2315.
31. Mazon, M. J., J. M. Gancedo, and C. Gancedo. 1982. Phosphorylation and inactivation of yeast fructose-bisphosphatase in vivo by glucose and by proton ionophores. A possible role for cAMP. *Eur. J. Biochem.* **127**:605-608.
32. McGrath, J., D. Capon, D. Goeddel, and A. Levinson. 1984. Comparative biochemical properties of normal and activated human *ras* p21 protein. *Nature (London)* **310**:644-655.
33. Neigeborn, L., P. Schwartzberg, R. Reid, and M. Carlson. 1986. Null mutations in the *SNF3* gene of *Saccharomyces cerevisiae* cause a different phenotype than do previously isolated missense mutations. *Mol. Cell. Biol.* **6**:3569-3574.
34. Nikawa, J., S. Cameron, T. Toda, K. M. Ferguson, and M. Wigler. 1987. Rigorous feedback control of cAMP levels in *Saccharomyces cerevisiae*. *Genes Dev.* **1**:931-937.
35. Powers, S., T. Kataoka, O. Fasano, M. Goldfarb, J. Strathern, J. Broach, and M. Wigler. 1984. Genes in *Saccharomyces cerevisiae* encoding proteins with domains homologous to the mammalian *ras* proteins. *Cell* **36**:607-612.
36. Purwin, C., K. Nicolay, W. A. Scheffers, and H. Holzer. 1986. Mechanism of control of adenylate cyclase activity in yeast by fermentable sugars and carbonyl cyanide *m*-chlorophenylhydrazone. *J. Biol. Chem.* **261**:8744-8749.
37. Robinson, L. C., J. B. Gibbs, M. S. Marshall, I. S. Sigal, and K. Tatchell. 1987. *cdc25*: a component of the *Ras*-adenylate cyclase pathway in *Saccharomyces cerevisiae*. *Science* **235**:1218-1221.
38. Sweet, R. W., S. Yokoyama, T. Kamata, J. R. Feramisco, M. Rosenberg, and M. Gross. 1984. The product of *ras* is a GTPase and the T24 oncogenic mutant is deficient in this activity. *Nature (London)* **311**:273-275.
39. Tatchell, K. 1986. *RAS* genes and growth control in *Saccharomyces cerevisiae*. *J. Bacteriol.* **166**:364-367.
- 39a. Thevelein, J. M. 1988. Regulation of trehalase activity by phosphorylation-dephosphorylation during developmental transitions in fungi. *Exp. Mycol.* **12**:1-12.
40. Thevelein, J. M., and M. Beullens. 1985. Cyclic AMP and the stimulation of trehalase activity in the yeast *Saccharomyces cerevisiae* by carbon sources, nitrogen sources and inhibitors of protein synthesis. *J. Gen. Microbiol.* **131**:3199-3209.
41. Thevelein, J. M., M. Beullens, F. Honshoven, G. Hoebeek, K. Detremmerie, J. A. den Hollander, and A. W. H. Jans. 1987. Regulation of the cAMP level in the yeast *Saccharomyces cerevisiae*: intracellular pH and the effect of membrane depo-

- larizing compounds. J. Gen. Microbiol. 133:2191-2196.
42. Thevelein, J. M., M. Beullens, F. Honshoven, G. Hoebeeck, K. Detremmerie, B. Griewel, J. A. den Hollander, and A. W. H. Jans. 1987. Regulation of the cAMP level in the yeast *Saccharomyces cerevisiae*: the glucose-induced cAMP signal is not mediated by a transient drop in the intracellular pH. J. Gen. Microbiol. 133: 2197-2205.
43. Toda, T., I. Uno, T. Ishikawa, S. Powers, T. Kataoka, D. Broek, S. Cameron, J. Broach, K. Matsumoto, and M. Wigler. 1985. In yeast, RAS proteins are controlling elements of adenylate cyclase. Cell 40:27-36.
44. Trahey, M., R. J. Milley, G. E. Cole, M. Innis, H. Paterson, C. J. Marshall, A. Hall, and F. McCormick. 1987. Biochemical and biological properties of the human N-ras p21 protein. Mol. Cell. Biol. 7:541-544.
45. Trevillyan, J. M., and M. L. Pall. 1979. Control of cyclic adenosine 3',5'-monophosphate levels by depolarizing agents in fungi. J. Bacteriol. 138:397-403.
46. Valle, E., L. Bergillos, S. Gascon, F. Parra, and S. Ramos. 1986. Trehalase activation in yeasts is mediated by an internal acidification. Eur. J. Biochem. 154:247-251.