FEBS Letters 356 (1994) 249–254

FEBS 14886

Cdc25 is not the signal receiver for glucose induced cAMP response in *S. cerevisiae*

Doron Goldberg, Marisa Segal, Alexander Levitzki*

Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received 10 October 1994; revised version received 8 November 1994

Abstract The Ras/cAMP pathway in the yeast *S. cerevisiae* couples the cell cycle of this unicellular organism to the availability of nutrients. Glucose derepressed *S. cerevisiae* cells respond to glucose addition by an intracellular rise in cAMP. In the prevailing model, yeast Ras plays a similar role to that of heterotrimeric G-proteins coupled to cell surface receptors. A crucial element of this model is that the exchanger, Cdc25 is activated by glucose. Such activation would result in a glucose-dependent rise in GTP-bound Ras concentration. We here show, in contrast to this view, that Cdc25 cannot be the receiver of the glucose signal. We suggest that the Ras-GTP/cyclase complex is the molecular element directly receiving the signal while Cdc25-dependent exchange constitutes a prerequisite for complex formation.

Key words: S.cerevisiae; Ras; Cdc25; Adenylyl cyclase; Cyclic adenosine monophosphate; Glucose response

1. Introduction

Mammalian Ras proteins are 21 kDa, small GTP binding proteins, attached to the inner leaflet of the plasma membrane, which play a pivotal role in transmission of growth stimulatory signals, differentiation and malignant transformation [1,21,29]. Ras cycles between an active GTP-bound and an inactive GDP-bound form. This process is regulated by guanine nucleotide exchange factors (GEFs), which catalyze GDP–GTP exchange, generating the active GTP-bound form, and GTPase activating proteins (GAPs), which stimulate the intrinsic Ras GTPase, promoting the hydrolysis to the inactive GDP-bound form. [5,6]

The mammalian Ras-mediated signal transduction system has a parallel in the yeast *S. cerevisiae* [15]. In this organism the two Ras proteins, Ras1 and Ras2, are components of a pathway which couples the activity of the enzyme adenylyl cyclase (Cdc35/Cyr1) to nutrient availability [36]. cAMP levels play a central role in the decision to progress through START, a control point at the G1/S boundary of the cell cycle after which cells become committed to the completion of mitosis. In addition, cAMP levels also determine the transition between fermentation and respiration.

Glucose-starved yeast cells and cells growing on a non-fermentable carbon source (glucose derepressed cells) respond to the addition of glucose by a 2- to 3-fold transient rise in intracellular cAMP levels [22,28] which is strictly dependent on the integrity of the Ras/adenylyl cyclase pathway [23,38]. Although the role of the glucose response is not entirely clear, it may signal the switch from respiration to fermentation since it is glucose repressible and appears only at the transition between these two modes of metabolism [2,36]. The glucose sensing system is believed to consist of the low affinity glucose transporter and at least one of three glucose phosphorylating enzymes [2], and is coupled to the cyclase enzyme by the Ras proteins and their regulators. These are Cdc25, the yeast Ras-GEF [7,8,10] and two proteins possessing GAP activity – Ira1 and Ira2 [33,35]. It has been proposed that yeast Ras mediates modulation of cyclase activity by glucose through a mechanism analogous to that by which heterotrimeric G-proteins mediate the regulation of mammalian cyclase by hormone-bound receptors. Glucose, therefore, has been postulated as a stimulator, whether directly or indirectly, of Cdc25 [26,38]. Upon glucose addition, Cdc25 activates Ras which in turn stimulates adenylyl cyclase.

Here we present evidence which suggests that, contrary to this notion, the glucose response depends primarily on the steady state concentration of the active, GTP-bound Ras and not on enhanced GDP–GTP exchange. Hence, the entity which responds to the influx of glucose is not the GEF itself but rather the cyclase/Ras-GTP complex. This view may have implications on the mode of action of Ras proteins in mammalian systems.

2. Materials and methods

2.1. Strains, media and genetic techniques

S. cerevisiae strains used in this study are listed in Table 1. All strains are isogenic to TT1A-4 (*MATa his3 leu2 ura3 trp1 ade8 cdc25::URA3*, pCDC25 [7]) kindly provided by M. Wigler. The level of expression of Cdc25 in each strain is shown in Fig. 1. Yeast cells were grown either in YEPD (1% yeast extract (Difco), 2% peptone (Difco), 2% dextrose) rich medium or in SD (0.67% yeast nitrogen base without amino acids (Difco), 2% dextrose) minimal medium supplemented with the required amino acids and nucleic acid bases (Sigma). Starvation medium was SD containing 0.02% dextrose instead of 2%. The permissive incubation temperature for thermosensitive strains was 23°C and the restrictive temperature was 37°C. Genetic techniques used were according to Sherman et al. [31].

2.2. Vectors and plasmids

Plasmids YEp21-CDC25, YCp403-CDC25 and pRS413-CDC25 contain the 5.4 kb *SalI-PvuII* fragment of the *CDC25* gene cloned into the vectors YEp21, YCp403 and pRS413, respectively [30]. pRS413 [32] was a gift from P. Hieter. pcdc25-2 is a multicopy plasmid carrying a *SalI-PvuII* fragment encoding the *cdc25-2*(ts) allele. Plasmid pNR2 [33], kindly provided by K. Matsumoto, was used for disruption of the *IRA1* gene. pHIS3-RAS2^{val19} was kindly provided by M. Wigler [19]. Plasmids pYCR and pYCΔ20 were a gift from J. Field [25]. pYCR is a multicopy plasmid carrying the complete yeast *CDC35* gene and pYCΔ20 is a multicopy plasmid carrying a truncated version of the gene lacking 201 bases at the 3′ end of the coding sequence.

*Corresponding author. Fax: (972) (2) 51-2958.

SSDI 0014-5793(94)01273-3

2.3. Glucose response and cAMP assay

Glucose addition to starved cells and cAMP assays were performed as previously described [16].

2.4. Adenylyl cyclase assay

Preparation of plasma membrane fractions and measurements of the kinetic parameters of adenylyl cyclase activation were performed according to Engelberg et al. [12].

2.5. Western blot analysis of Cdc25

Analysis of Cdc25 by Western blot was performed essentially as previously described by Gross et al. [18].

3. Results

3.1. Operational definition of zero-response

In order to make a clear distinction between weak responses to glucose of starved cells, which we encountered during this study, and no response at all, we sought an operational definition for a zero-response. We therefore, constructed an isogenic cdc25^{ts} strain carrying pcdc25-2, which harbored the *cdc25-2*(ts) allele (SMR1-TS). Glucose response in this strain at the restrictive temperature was taken as zero-response (Fig. 2). The response was characterized by the absence of any increase of the cAMP concentration above basal levels (prior to the addition of glucose) even after 5 min. A genuine response was then defined as the rise in cAMP concentration, following addition of glucose, above the levels under starvation. The positive control in this series of experiments was strain SMR1-21 which harbored a centromeric plasmid encoding the wild type Cdc25. Glucose response in this strain (Fig. 2) was similar to that of wild type strains both in its extent and overall pattern [26,38].

3.2. Lack of correlation between Cdc25's exchange capability and the rate of cAMP accumulation during the glucose response

The pseudo-first-order rate constant of the GDP/GTP exchange reaction on Ras proteins (κ_{obs}) can be inferred from measurements of the kinetics of adenylyl cyclase activation in the presence of Mg^{2+} and Gpp[NH]p [37]. It has been shown that the measured κ_{obs} in plasma membranes prepared from cdc25^{ts} cells was 4-fold smaller than the measured κ_{obs} in plasma membranes prepared from cells having the intact, wild type protein [12].

Kinetic experiments were performed with plasma membranes prepared from two isogenic strains – SMR1-TS, which harbored the *cdc25-2*(ts) allele, and SMR1-22, which contained the wild type *CDC25* gene. Both genes were expressed from high copy number plasmids (see Table 1). The κ_{obs} values measured were 0.04 min^{-1} and 0.13 min^{-1}, respectively, in agreement with previously reported values. This difference may partly emanate from the lower stability of the mutant protein which results in its decreased concentration (Fig. 1). However, when we measured the glucose response in these two strains at the permissive temperature (23°C) we found no difference in the rate of cAMP accumulation (Fig. 3). It should be noted that the response of strain SMR1-22 (overexpressing wild type Cdc25) lacked its descending phase (see also below).

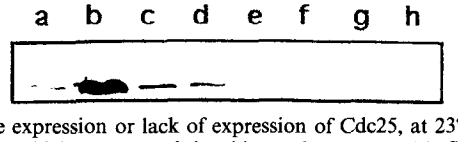

Fig. 1. The expression or lack of expression of Cdc25, at 23°C, in all the strains which were used in this study. Lanes: (a) SMR1-21; (b) SMR1-22; (c) SMR1-TS; (d) SMR1-24; (e) SMR1-23; (f) SMR1-25; (g) SMR1-31; (h) SMR1-32.

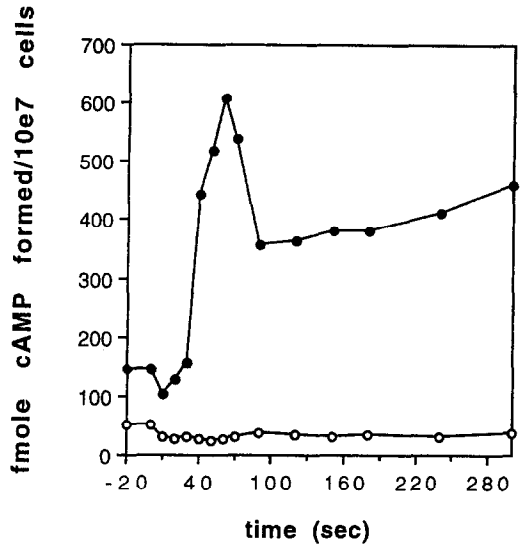

Fig. 2. The glucose response at 30°C of strain SMR1-21 having a wild type Cdc25 expressed from a centromeric plasmid (●) and the glucose response of the temperature sensitive strain SMR1-TS, harboring the cdc25-2(ts) allele on a multicopy plasmid, at the restrictive temperature (37°C) (○). Glucose addition is marked by an arrow.

This phenomenon, taken together with the fact that the measured κ_{obs} values in plasma membranes of yeast cells were always high, even without stimulation [12], has led us to speculate that the glucose-induced cAMP response might not be dependent on enhancement of the exchange reaction, taking place upon addition of glucose, but rather on the steady-state levels of the active complex of GTP-bound Ras and adenylyl cyclase.

Table 1
Yeast strains used in this study

Strain	Genotype
SMR1-21	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3,* YCp403-CDC25[*CDC25*, CEN, *HIS3*].
SMR1-22	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3,* YEp21-CDC25[*CDC25, 2μ, LEU2*].
SMR1-23	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3, iral:LEU2,*
SMR1-24	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3, iral:LEU2,* pRS413-CDC25[*CDC25*, CEN, *HIS3*].
SMR1-25	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3, his3::pHIS3-RAS2^{val19}:*
SMR1-TS	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3,* pcdc25^{ts}[*cdc25-2, 2μ, HIS3*].
SMR1-31	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3,* pYCR[*CDC35, 2μ, LEU2*].
SMR1-32	*MATa, his3, leu2, ura3, trpl, ade8, cdc25::URA3,* pYC*Δ*20[*cdc35-Δ20, 2μ, HIS3*].

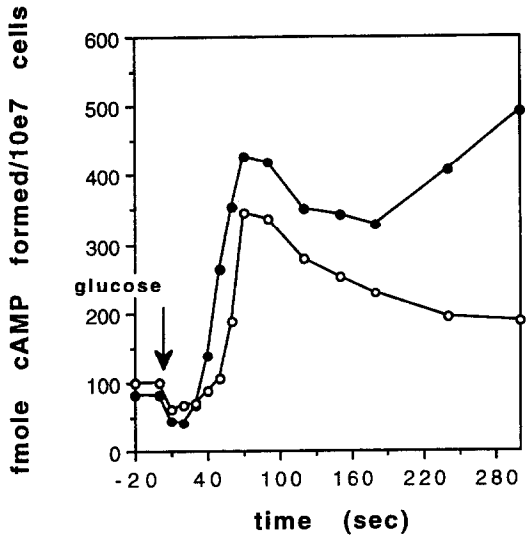

Fig. 3. The glucose response, at 23°C, of the strains SMR1-TS (cdc25^{ts}) (○) and SMR1–22 (overexpressing wild type Cdc25) (●).

3.3. Glucose response in a cdc25Δ strain carrying the *RAS2^{val19}* gene

According to the hypothesis suggesting that the glucose response depends primarily on the steady state concentrations of the GTP-bound Ras and not on an enhanced exchange reaction, the presence of the GEF could be dispensed with, provided that the steady state concentration of active Ras proteins was maintained at high enough level by means of appropriate genetic manipulations of the cAMP pathway.

We, therefore, constructed an isogenic cdc25Δ strain carrying an integrated *RAS2^{val19}* gene (SMR1-25). This mutated RAS2 encodes a protein which is constitutively active even in the absence of Cdc25 [7]. We compared the glucose response of strain SMR1-25 to that of the cdc25^{ts} strain (SMR1-TS) and strain SMR-22 (overexpressing wild type Cdc25). The strain expressing *RAS2^{val19}* exhibited a clear glucose response both at 30°C and the restrictive (37°C) temperature (Fig. 4). Yet the cAMP levels under starvation were higher than those in strain SMR1-22 but the profile of the response was similar and lacked the descending portion of the peak.

Our results differ from a previous report by Van Aelst et al. who showed only a marginal response in a cdc25Δ strain expressing *RAS2^{val19}* [38]. The reason for this discrepancy is not clear.

3.4. Disruption of the IRA1 gene restored the glucose response in a cdc25Δ strain

To further test the hypothesis that the glucose response is dependent on a certain steady state level of GTP-bound Ras rather than on enhanced exchange, we assayed the glucose response of strain SMR1-24 (*cdc25::URA3, ira1::LEU2*). The viability of this cdc25Δ strain was restored by disruption of the *IRA1* gene. Under such conditions higher proportions of Ras proteins remain in their GTP-active state [34]. This was confirmed by measurements of cyclase activity in vitro (not shown). Strain SMR1-24 exhibited a glucose response (Fig. 5) which was similar to that of strain SMR1-25 (*RAS2^{val19}*) (Fig. 4) since it lacked the descending portion of the peak, though the increase in cAMP was less pronounced. This result agrees with a previous report by Tanaka et al. [33] who also showed that a strain with a deleted *CDC25* and IRA1 genes exhibited a glucose response. Reintroducing the *CDC25* gene on a centromeric plasmid in this strain did not change the general pattern of the glucose response but the overall levels of cAMP were higher (Fig. 5).

3.5. The glucose response can be restored in a cdc25Δ strain by overexpression of a Ras-independent adenylyl cyclase

If the glucose response is dependent on the formation of the active complex, Ras-GTP/cyclase, then the role of Ras may be to induce a conformational change on adenylyl cyclase to render it glucose responsive. We speculated that particular cyclase mutant proteins might assume the conformation required for responsiveness to glucose, in a Ras-independent manner. If this were the case, such mutants would display a glucose response irrespective of the presence of active Ras proteins. To test this hypothesis we transformed a cdc25Δ strain with a multicopy plasmid pYCΔ20 carrying a truncated cyclase coding for a protein lacking 67 amino acids of its C-terminus (strain SMR1-32). Such a truncation abolishes both binding of the cyclase associated protein (Cap) and interaction with Ras [25]. The Ras-independent activity of the enzyme was confirmed by cyclase measurements (not shown). This Ras-independent cyclase might be capable of more easily assuming the glucose responsive conformation than a wild type Ras-dependent cyclase.

Indeed strain SMR1-32 responded to the addition of glucose (Fig. 6) although the response was late and its extent was relatively small. However, the response is clearly evident when compared to that of a control strain (SMR1-31) harboring the complete adenylyl cyclase gene on high copy number plasmid.

3.6. The identity of the response stimulator

An intriguing aspect of the glucose response phenomenon is whether glucose itself or a glucose metabolite constitutes the

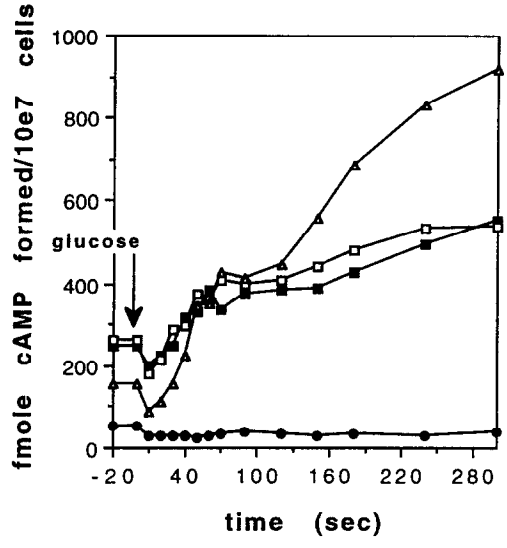

Fig. 4. The glucose response of strain SMR1-25 harboring an integrated *RAS2^{val19}* gene at 30°C (□) and at the restrictive temperature (37°C) (■) compared to that of strain SMR1-TS (cdc25^{ts}) at the restrictive temperature (●) and strain SMR1-22 (overexpressing wild type Cdc25) at 30°C (△).

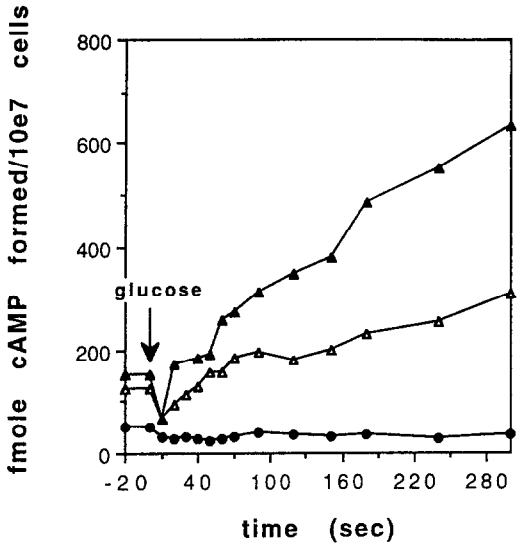

Fig. 5. The glucose response of the strains with a disrupted *IRA1* gene – SMR1–23 (△) and SMR1-24 (▲) at 30°C compared to the zero-response of strain SMR1-TS (cdc25^{ts}) at the restrictive temperature (37°C) (●).

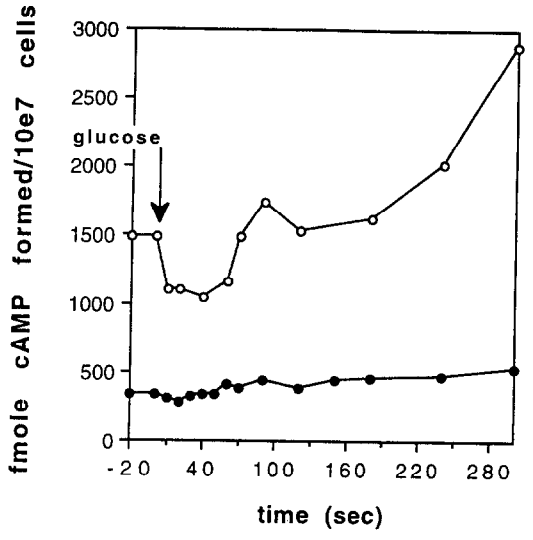

Fig. 6. The glucose response, at 30°C, of strain SMR1-31 overexpressing a normal adenylyl cyclase (●) and strain SMR1-32 overexpressing a truncated, Ras-independent, enzyme (○).

ligand for the glucose sensing mechanism. We tested the response of strain SMR1-21 to the addition of xylose and 6- deoxyglucose. Both sugars can be transported into the cells but are not phosphorylated [4,13]. In contrast to previously reported results [13], xylose did not stimulate any response while 6-deoxyglucose stimulated only a negligible increase in intracellular cAMP concentration (Fig. 7). Furthermore, we have confirmed (not shown) that yeast cells lacking all three glucose phosphorylating enzymes (two hexokinases and a glukokinase) were incapable to respond to glucose, since the available data were inconsistent [2,14]. Although high affinity glucose uptake is also impaired in such triple mutants [3], the high K_m (25 mM) of the glucose response [2] suggests that high affinity uptake is not involved in this phenomenon.

Since no metabolism beyond sugar phosphorylation is essential for the glucose response [2], these two observations point to the possibility that the stimulator of the response is either glucose phosphorylated at its sixth position or some non-glycolytic metabolic product of this sugar.

4. Discussion

It has been suggested that the role of Ras in the modulation of the yeast adenylyl cyclase is analogous to that of mammalian heterotrimeric G-proteins. Much of the data that has accumulated over the last few years on this model system seems to support this notion [2,11]. According to this model, Cdc25, the yeast Ras exchanger, may sense the extracellular signals, such as glucose, and respond by enhancing the rate of the GDP–GTP exchange on Ras. The GTP-bound Ras, in turn, activates the cyclase. This sequence of events result in higher cAMP levels [36].

Here, we have presented data which cannot be accommodated by this model. We have shown that it is possible to maintain the responsiveness of the yeast adenylyl cyclase to the influx of glucose in the complete absence of the GEF – Cdc25 (Figs. 4 and 5) and that the rate of cAMP accumulation during the glucose response is not dependent on the rate of Cdc25-catalyzed exchange reaction as was measured by our kinetic in vitro assay (Fig. 3). Moreover, we have shown that cells overexpressing Ras-independent adenylyl cyclase mutant protein can also respond in the absence of Cdc25 and active Ras proteins (Fig. 6).

The glucose response in strains, lacking Cdc25, in which active Ras concentrations are held constantly high by second site mutations, is similar to that of a strain overexpressing wild type Cdc25, both in extent and time course (Fig. 4). It is not possible to attribute this response to the presence of a second GEF, Sdc25 [9] since *CDC25* is indispensable for viability while Sdc25 is not expressed under normal conditions, including the conditions used in this study [9].

The different profile of the response, namely the lack of the

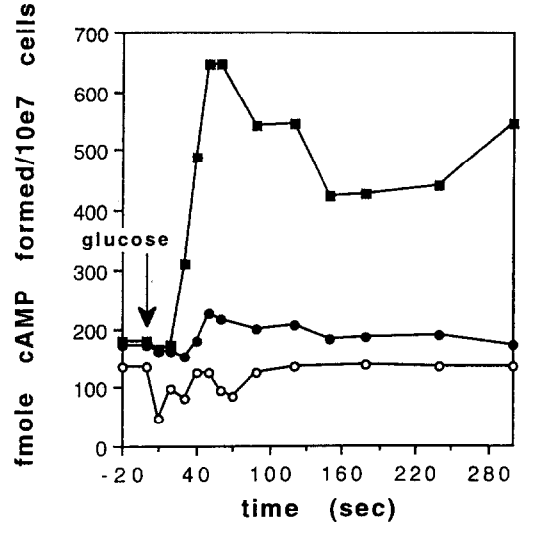

Fig. 7. The response, at 30°C, of strain SMR1-21 (Cdc25 expressed from a centromeric plasmid) to the addition of 100 mM xylose (○), 50 mM 6-deoxyglucose (●), 50 mM glucose (■).

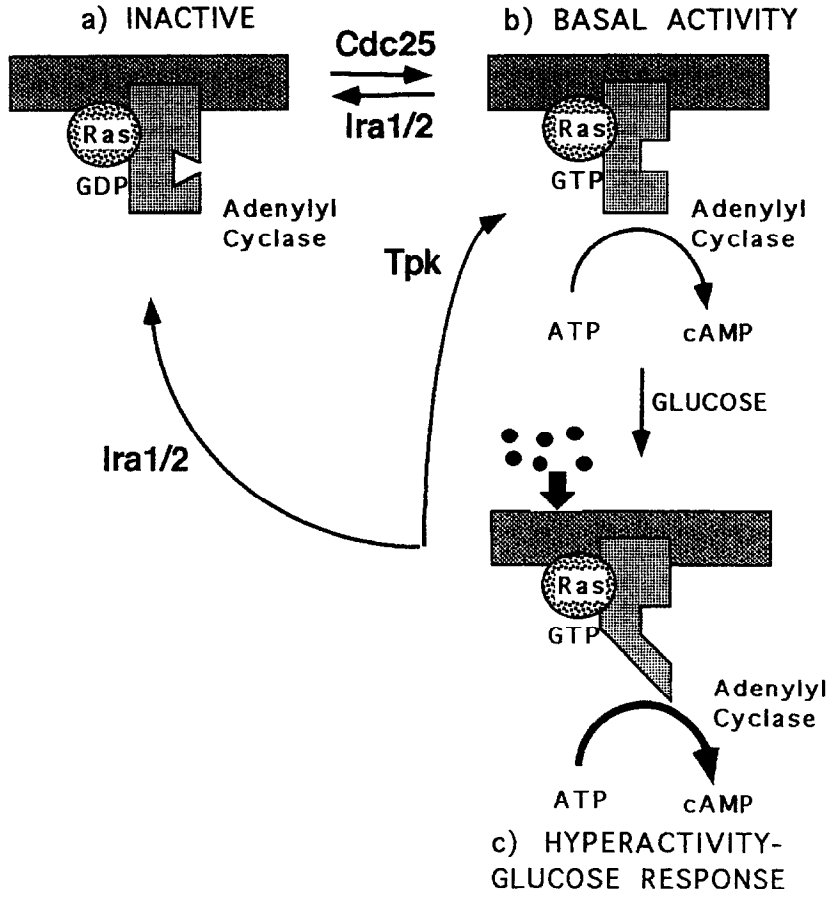

Fig. 8. A new model of the adenylyl cyclase system of the yeast *S. cerevisiae*. The cyclase system, in glucose-starved cells, can oscillate between an inactive (a) and basally active (b) states which are determined by the occupancy of Ras with GTP. This process is controlled by the combined action of Cdc25 and the Ira proteins. When Ras is in its GTP bound form, the cyclase system is basally active and can respond to the entry of glucose. Once glucose flows into the cell, the system shift to an hyperactive mode (c) and glucose response occurs. The system can exit the hyperactive state by the action of the GAP proteins which render it inactive or can be downregulated by the action of the cAMP dependent protein kinase (see section 4).

descending portion of the cAMP peak observed in strains which possess mutations such as $RAS2^{val19}$ or a deleted *IRA1* gene (Figs. 4 and 5), was also observed in the strain overexpressing Cdc25 (Fig. 4). This phenomenon was previously described in tpk^{w} yeast strains with a weakened activity of the catalytic subunit of he cAMP-dependent protein kinase [24,27]. It is possible that the two phenomena are related. One plausible explanation is that cAMP levels, under normal conditions, fall sharply after reaching their peak when the cyclase system is inhibited by phosphorylation of its components by the cAMP-dependent protein kinase as part of an inhibitory feedback loop [17]. Mutations, as mentioned above, can generate high levels of active adenylyl cyclase which would overcome this inhibition and result in cAMP concentrations that eventually level-off but do not go down.

The discrepancy between the prevailing model and our findings can be reconciled by an alternative model (Fig. 8). In this model, the Ras-GTP/adenylyl cyclase complex is the molecular entity which senses the entry of glucose into the cell. This is done, most likely, by responding to elevated concentration of glucose 6-phosphate or a product of its metabolism. The role of Ras proteins in this model is to control the basal activity of adenylyl cyclase and, in addition, to monitor the sensitivity of the enzyme to the influx of glucose into the cell. The steady state concentration of the GTP-bound Ras is, therefore, the prime factor which governs the extent of the glucose response. This steady-state is regulated by the combined action of Cdc25 and the two Ira proteins, which would not be an integral part of the glucose sensing system. Low steady-state concentrations of GTP-bound Ras would result in an insensitive cyclase and loss of the glucose response. On the other hand, concentrations of active Ras above a certain threshold should enable the enzyme to respond when glucose flows in (Fig. 8). Our finding that a Ras-independent cyclase can respond (Fig. 6) is in accordance with this model.

Additional modulation of the system following activation, may still operate through Cdc25 as a result of phosphorylation by cAMP-dependent protein kinase as was demonstrated in our previous study [17].

This model addresses solely the mechanism underlying the glucose response and not the biological significance of this phenomenon. It is also uncertain whether this model has any bearing on the mechanism by which extracellular signals are mediated by Ras proteins in higher eukaryotes. Yet, given the excellent record of yeast as a model system, we may still learn from the glucose paradigm about the relationship between Ras

and its effectors in mammalian cells. It has been shown [20,39,40] that Ras by itself does not activate its downstream effector Raf, but probably marks it for activation by other molecules. At least in this respect, the mammalian system resembles our model in which Ras proteins render the cyclase system responsive to the entry of glucose but do not in themselves relay the extracellular signal to the enzyme.

References

[1] Barbacid, M. (1987) Annu. Rev. Biochem. 56, 779–827.
[2] Beullens, M., Mbonyi, K., Greets, L., Gladines, D., Detremerie, K., Jans, A.W.H. and Thevelein, J.M. (1988) Eur. J. Biochem. 172, 227–231.
[3] Bisson, L.F. and Fraenkel, D.G. (1983) Proc. Natl. Acad. Sci. USA 80, 1730–1734.
[4] Bisson, L.F. and Fraenkel, D.G. (1983) J. Bacteriol. 155, 995–1000.
[5] Boguski, M.S. and McCormick, F. (1993) Nature 366, 643–654.
[6] Bollag, G. and McCormick, F. (1991) Annu. Rev. Cell Biol. 7, 601–632.
[7] Broek, D., Toda, T., Michaeli, T., Levin, L., Birchmeier, C., Zoller, M., Powers, S. and Wigler, M. (1987) Cell 48, 789–799.
[8] Camonis, J.H., Kalekine, M., Gondre, B., Garreau, H., Boy-Marcotte, E. and Jacquet, M. (1986) EMBO J. 5, 375–380.
[9] Damak, F., Boy-Marcotte, E., Le-Roscouet, D., Guilbaud, R. and Jacquet, M. (1991) Mol. Cell. Biol. 11, 202–212.
[10] Daniel, J. and Simchen, G. (1986) Curr. Genet. 10, 643–646.
[11] Engelberg, D., Perlman, R. and Levitzki, A. (1989) Cell. Sig. 1, 1–7.
[12] Engelberg, D., Simchen, G. and Levitzki, A. (1990) EMBO J. 9, 641–651.
[13] Eraso, P. and Gancedo, J.M. (1985) FEBS Lett. 191, 51–54.
[14] Eraso, P., Mazùn, M.J. and Gancedo, J.M. (1987) Eur. J. Biochem. 165, 671–674.
[15] Gibbs, J.B. and Marshall, M.S. (1989) Microbiol. Rev. 53, 171–185.
[16] Goldberg, D., Marbach, I., Gross, E., Levitzki, A. and Simchen, G. (1993) Eur. J. Biochem. 213, 195–204.
[17] Gross, E., Goldberg, D. and Levitzki, A. (1992) Nature 360, 762–765.
[18] Gross, E., Marbach, I., Engelberg, D., Segal, M., Simchen, G. and Levitzki, A. (1992) Mol. Cell. Biol. 12, 2653–2661.
[19] Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J. and Wigler, M. (1984) Cell 37, 437–445.
[20] Kolch, W., Heidecker, G., Kochs, G., Hummel, R., Vahidi, H., Mischak, H., Finkenzeller, G., Marmé, D. and Rapp, U.R. (1993) Nature 364, 249–252.
[21] Lowy, D.R. and Willumsen, B.M. (1993) Annu. Rev. Biochem. 62, 851–891.
[22] Mazùn, M.J., Gancedo, J.M. and Gancedo, C. (1982) Eur. J. Biochem. 127, 605–608.
[23] Mbonyi, K., Beullens, M., Detremerie, K., Greerts, L. and Thevelein, J.M. (1988) Mol. Cell. Biol. 8, 3051–3057.
[24] Mbonyi, K., Van Aelst, L., Argüelles, J.C., Jans, A.W.H. and Thevelein, J.M. (1990) Mol. Cell. Biol. 10, 4518–4523.
[25] Mintzer, K.A. and Field, J. (1994) Cell. Sig. (in press).
[26] Munder, T. and Küntzel, H. (1989) FEBS Lett. 242, 341–345.
[27] Nikawa, J.-i., Cameron, S., Toda, T., Ferguson, K.M. and Wigler, M. (1987) Genes Dev. 1, 931–937.
[28] Purwin, C., Leidig, F. and Holzer, H. (1982) Biochem. Biophys. Res. Commun. 107, 1482–1489.
[29] Satoh, T., Nakafuku, M. and Kaziro, Y. (1992) J. Biol. Chem. 267, 24149–24152.
[30] Segal, M., Willumsen, B.M. and Levitzki, A. (1993) Proc. Natl. Acad. Sci. USA 90, 5564–5568.
[31] Sherman, F., Fink, G.R. and Hicks, J.B. (1986) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[32] Sikorski, R.S. and Hieter, P. (1989) Genetics 122, 19–27.
[33] Tanaka, K., Matsumoto, K. and Toh-e, A. (1989) Mol. Cell. Biol. 9, 757–768.
[34] Tanaka, K., Nakafuku, M., Satoh, T., Marshall, M.S., Gibbs, J.B., Matsumoto, K., Kaziro, Y. and Toh-e, A. (1990) Cell 60, 803–807.
[35] Tanaka, K., Nakafuku, M., Tamanoi, F., Kaziro, Y., Matsumoto, K. and Toh-e, A. (1990) Mol. Cell. Biol. 10, 4303–4313.
[36] Thevelein, J.M. (1991) Mol. Microbiol. 5, 1301–1307.
[37] Tolkovsky, A.M. and Levitzki, A. (1978) Biochemistry 17, 3795–3810.
[38] Van Aelst, L., Jans, A.W.H. and Thevelein, J.M. (1991) J. Gen. Microbiol. 137, 341–349.
[39] Vojtek, A.B., Hollenberg, S.M. and Cooper, J.A. (1993) Cell 74, 205–214.
[40] Zhang, X.-f., Settleman, J., Kyriakis, J.M., Takeuchi-Suzuki, E., Elledge, S.J., Marshall, M.S., Bruder, J.T., Rapp, U.R. and Avruch, J. (1993) Nature 364, 308–313.