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The *pde2* gene of *Saccharomyces cerevisiae* is allelic to *rca1* and encodes a phosphodiesterase which protects the cell from extracellullar cAMP

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The high affinity cAMP phosphodiesterase, encoded by *PDE2*, is an important component of the cAMP-dependent protein kinase signaling system in *Saccharomyces cerevisiae* An unexpected phenotype of *pde2* mutants is sensitivity to external cAMP. This trait has been found independently for *rca1* mutants and has been used to monitor the effects of cAMP on several biological processes. We demonstrate here that *RCA1* is identical to *PDE2*. Further analysis of the phenotype of *pde2* deletions reveal that exogenously added cAMP results in an increase in the internal level of cAMP. This increase slows down the rate of cell division by increasing the length of the G_1 phase of the cell cycle and leads to increased cell volume. Also, cells with a disrupted *PDE2* gene previously arrested by nutrient starvation rapidly lose thermotolerance when incubated with exogenous cAMP. From these observations we propose that a role of the *PDE2*-encoded phosphodiesterase may be to help insulate the internal cAMP pools from the external environment This protective role might also be important in other eukaryotic organisms where cAMP is a key second messenger.

Cyclic AMP; Phosphodiesterase; Yeast; Growth control

1. INTRODUCTION

In *Saccharomyces cerevisae*, cAMP is a second messenger that modulates the activity of cAMP-dependent protein kinase, which in turn influences many biological processes. The activity of cAMP-dependent protein kinase regulates carbohydrate metabolism and storage, is involved in nitrogen metabolism, controls cell growth and division and, regulates mitochondrial functions and responses to nutritional and heat stresses (reviewed by Broach and Deschenes [1]). The activity of the adenylyl cyclase, encoded by *CDC35/CYR1*, is regulated by two Ras proteins. Ras is itself under the positive control of the *CDC25* gene product, which acts as a guanine nucleotide exchange factor, and under negative control by the *IRA1* and *IRA2* gene products, which enhance the GTPase activity of Ras. Although the signal or signals mediated by Ras are not well understood glucose has been shown to modulate the activity of adenylyl cyclase [1].

Two cAMP phosphodiesterases have been identified in yeast. One with low affinity is encoded by *PDE1* [2] and the other with high affinity is encoded by *PDE2/SRA5* [3,4]. Defects in either or both of these, however, do not lead to a dramatic increase in cAMP, and under most conditions the *pde* mutants have nearly a wild-type phenotype. The insensitivity of cAMP levels to mutations in *pde1* and *pde2* is primary evidence for a strong feedback control mechanism exercised by cAMP-dependent protein kinase on cAMP levels [5]. The precise mechanism of the feedback control is not well understood, but both Ras [6] and Cdc25 [7] have been shown to be phosphorylated in vivo.

Mutations in *pde2* allowed cells lacking both *ras1* and *ras2* to grow on rich media in the presence of exogenous cAMP [4]. This unexpected phenotype lead us to further investigate the role of the high-affinity phosphodiesterase in the cellular response to exogenous cAMP. The same phenotype has been reported for *rca1*, a recessive mutation allowing cAMP to rescue the growth defect of *cdc25* and *cdc35* mutations [8,9]. In this report we prove that *PDE2* and *RCA1* are the same gene. We show that in contrast to wild-type strains, the level of internal cAMP is increased in *pde2* strains after the addition of external cAMP. We also demonstrate that the addition of cAMP to *pde2* strains slows the division rate and results in a larger cell size. Strains previously arrested by nutritional stress become heat-shock sensitive when exposed to exogenous cAMP. These results lead us to propose that one role for the high-affinity phosphodiesterase could be to protect the cAMP signaling pathway from interference by the extracellular environment which would alter growth and metabolism.

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2. MATERIALS AND METHODS

2.1. *Yeast strains*

Haploid stdrains RW300 (MATα) and RW301 (MATα trp1 pde2::TRP1) are isogenic and were derived from RW245-18A (*MATα trp1*) by transformation. RW245-18A was transformed with *TRP1* on the 1.45 kb *Eco*RI fragment of plasmid pJH-W1 (kindly provided by John Hill) to obtain RW300. All 88 spores from 22 tetrads derived from a cross between RW300 and RW257 (*MAT***a** *leu2 ade2 his4 lys2 ras2-530*) were Trp$^+$, indicating that the *TRP1* gene in RW300 integrated at the *TRP1* locus. *PDE2* was disrupted by transforming RW245-18A with the 6.0 kb *Bam*HI *Sst*I fragment of pW6 [4], which contains the *TRP1* gene inserted at the *Eco*RI site in the open reading frame of *PDE2*, to obtain RW301. The restriction map of *pde2::TRP1* (formerly *sra5::TRP1*), derived by Southern analysis of RW301, is consistent with replacement of the wild-type allele by the disruption (data not shown). In addition, all 71 spores derived from a cross between RW301 and RW149-15B (*MAT***a** *trp1 ura3 leu2 lys2 pde2::TRP1*) were Trp$^+$ and displayed the phenotypes previously described for *PDE2*-disrupted strains [4]. OL520-1 (*MATα cdc25-5 rca1 ura3 leu2 his3 trp1*) results from a cross between OL97-1-11B (*MATα cdc25-5 ura3 leu2 his3 his7*) and OL512-2A (*MAT***a** *rca1 ura3 leu2 his3 trp1*). OL521-3 (*MAT***a** *cdc25-5 ade1(and/or)2 his7 arg4 trp1 pde2::TRP1*) results from a cross between D12BR19 (*MAT***a** *cdc25-5 ade1 arg4 his7 trp1*) and RW301.

2.2. *Media*

Complete rich medium (YEPD) contained 1% yeast extract, 2% Bacto-Peptone, and 2% glucose; complete minimal medium (MIN) and minimal nitrogen-free medium (MIN−N) have been described previously [10]. The effect of cAMP was followed by adding 4 mM cAMP to the different media Suppression of the thermosensitive phenotype of the *cdc25-5* allele by cAMP was tested at 36°C.

2.3. *DNA manipulations*

Procedures for DNA manipulations [11], yeast transformations [12], and yeast DNA preparation [13], have been described previously.

2.4. *Cell counts and size determinations*

Cell counts and size determinations were made with a Coulter Counter model ZM and Channelyzer model 256 (Coulter Electronics) after brief sonication to dissociate cell aggregates.

2.5. *Heat shock*

Cells grown to mid-log phase (1×10^7 cells/ml) in MIN medium at 30°C with shaking were transferred to an equal volume (2 ml) of prewarmed MIN medium at 48°C in a rotary-bath shaker. Samples were removed at various times into ice-cold sterile water. After brief sonication to dissociate cell aggregates, appropriate dilutions were plated on YEPD agar and incubated at 30°C for 2–4 days. For heat shock of nutrient-arrested cells, mid-log phase cultures grown in MIN medium at 30°C with shaking were washed twice with MIN−N and resuspended in MIN−N at 30°C in a rotary-bath shaker. After 2 days, cells were resuspended in either MIN−N or MIN−N+4 mM cAMP (Boehringer-Mannheim Biochemicals) at 30°C in a rotary-bath shaker; 6 h later, 1 ml was transferred to an equal volume of the same medium prewarmed to 48°C in a rotary-bath shaker Samples were removed and viabilities determined as above for log-phase heat shock.

2.6. *cAMP determinations*

Cells grown to mid-log phase in MIN medium at 30°C with shaking were suction-filtered on glass filters, rapidly washed with cold MIN+0.1 NaCl, and suspended in 3 ml of 0.5 M perchloric acid. The cells were disrupted by vortexing in the presence of glass beads, followed by centrifugation to remove insoluble material. The isolation of cAMP from the supernatant was performed using the alumina-dowex purification system [14,15]. cAMP content was determined by the modified radioimmunoassay procedure of Harper and Brook [16].

For determinations of cAMP concentrations in nutrient-arrested cells, mid-log phase cultures grown in MIN medium at 30°C with shaking were washed twice with MIN−N and resuspended in MIN−N at 30°C with shaking. After 2 days, the cells were resuspended in either MIN−N or MIN−N+4 mM cAMP at 30°C with shaking; 6 h later, the cells were suction filtered on glass filters, rapidly washed with cold MIN+0.1 M NaCl, and suspended in 0.5 M perchloric acid. The cells were disrupted, and cAMP content was determined as described above for log-phase cells. Protein concentrations were determined by centrifuging an aliquot from the cultures used for cAMP determinations, washing once with water, and resuspending in a buffer composed of 2 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl, pH 6.8, 10 mM $MgCl_2$, and 1 mM 2-mercaptoethanol. The cells were disrupted by vortexing in the presence of glass beads, followed by centrifugation to remove insoluble material. Protein concentrations in the supernatant were determined by the method of Bradford [17], with bovine serum albumin as the standard

2.7. *Determination of budded and unbudded cells*

Small aliquots of cultures were briefly sonicated to dissociate cell aggregates and examined under a microscope. To determine the proportion of unbudded cells at least 600 cells were examined.

2.8. *Determination of G_1 length*

The length of the G_1 period was calculated from mid-log phase cultures at 30°C with shaking, according to the equation described by Rivin and Fangman [18]: $G_1 = T_D\{1-\log(2-F_{unbud})/\log 2\}$, in which T_D is the mean doubling time and F_{unbud} is the fraction of unbudded cells.

3. RESULTS

3.1. *Allelism between* PDE2 *and* RCA1

A number of mutant yeast strains have been isolated in which exogenous cAMP exerts a pronounced biological effect. The *cam* strains isolated by Matsumoto et al. [19] are affected by μM cAMP concentrations and are demonstrably permeable to cAMP, however, the increased permeability phenotype is polygenic and is therefore difficult to transfer to other strain backgrounds. *pde2* [4] and *rca1* [8] mutations have also been reported to allow exogenous cAMP to exert physiological effects. To determine allelism between *pde2* and *rca1* we constructed two strains containing, respectively, the *rca1* mutation and the *pde2::TRP1* gene disruption in the presence of the *cdc25-5* mutation. OL520-1 (*rca1 cdc25-5*) and OL521-3 (*pde2::TRP1 cdc25-5*) are unable to grow at 37°C, the restrictive temperature for the *cdc25-5* mutation, but can be rescued by exogenously added cAMP. The diploid strain resulting from the mating between these two strains is thermosensitive and can also be rescued by cAMP, in contrast to diploid strains homozygous for *cdc25-5* but heterozygous for *rca1* or *pde2::TRP1*. *rca1* and *pde2* are therefore in the same complementation group. Allelism between *rca1* and *pde2* is further supported by close genetic linkage between the two mutations, as shown by the analysis of the progeny from seven tetrads produced by sporulation of the *rca1/pde2* diploid. All ascosporal clones were thermosensitive but were able to grow at the restrictive temperature in the presence of cAMP.

3.2. *Exogenous cAMP raises intracellular cAMP levels in* pde2 *strains*

The addition of cAMP to the media of *pde2/rca1*

Table I

Effects of exogenous cAMP on intracellular cAMP levels[a]

Strain	Medium	Supplement	
		None	4 mM cAMP
RW300[b]	MIN	2.1[d]	1.4[d]
RW301[b]	MIN	0.9[d]	57.2
RW301[c]	MIN-N	3.4[d]	51.3

[a] Levels were determined as described in section 2 and are expressed as 10^{-10} M cAMP per mg soluble protein.
[b] The RW300 (*PDE2*) and RW301 (*pde2*) cells were in mid-log phase.
[c] Mid-log phase RW301 (*pde2*) cells were arrested in MIN-N for 2 days after which cAMP was added for 6 h.
[d] These levels are not significantly different from each other

strains causes physiological changes similar to those caused by mutation that increase the activity of cAMP-dependent protein kinase. The most obvious explanation for these effects is that exogenous cAMP raises intracellular cAMP levels in *pde2/rca1* mutants. We therefore measured intracellular cAMP levels in exponentially growing cultures of RW301 (*MATα trp1 pde2::TRP1*), and the congenic wild-type RW300 (*MAT***a**), by radioimmunoassay (see section 2). The intracellular levels of cAMP in the wild-type strain (RW300) were not significantly affected by the addition of 4 mM cAMP to the medium (Table I). In the *pde2::TRP1* strain (RW301), however, 4 mM exogenous cAMP significantly raised intracellular cAMP levels (Table I). Exogenous cAMP also raised intracellular cAMP levels in RW301 (*pde2::TRP1*) cells previously arrested for 2 days by nitrogen starvation (see section 2). Six hours after 4 mM cAMP was added to the medium, intracellular cAMP levels were more than 15-fold higher than in control cells (Table I).

3.3. *Elevated intracellular cAMP levels prolong G_1 and increase cell volume, dry weight and protein content*

We measured the doubling times of RW300 (*PDE2*) and RW301 (*pde2::TRP1*) in complete minimal medium (MIN) with and without 4 mM cAMP. The doubling time of RW300 was not affected by the addition of 4 mM cAMP. In contrast, the doubling time of RW301 (*pde2::TRP1*) increased from 3.8 to 4.4 h with the addition of 4 mM cAMP to the media (Table II). By determining the fraction of unbudded cells in the cultures, we found that the increased doubling time of RW301 (*pde2*) in MIN+4 mM cAMP was due to an increase in the length of the G_1 phase of the division cycle (Table II, see section 2). The effects of exogenous cAMP are probably not due to increases in intracellular AMP. When we substituted 4 mM AMP for 4 mM cAMP, doubling times of both RW300 and RW301 were unchanged from those in MIN medium alone (data not shown).

We determined the diameter, dry weight, and protein content of mid-log phase RW300 (*PDE2*) and RW301 (*pde2*) cells in MIN medium, with and without 4 mM cAMP (see section 2). All three parameters of growth were significantly increased in RW301 (*pde2*) cells in MIN+4 mM cAMP (Table II). Dry weight and protein content of the *pde2* cells in the presence of 4 mM cAMP increased by over 100%.

The elevated levels of intracellular cAMP in the *pde2* strains could be due to either increased uptake of cAMP conferred by the *pde2* mutant, increased stability of the cAMP, or stimulation of adenylate cyclase by exogenous cAMP. We have measured cAMP uptake in RW300 and RW301 in MIN medium and observed no measurable difference in uptake under the conditions we used. From this we can only conclude that the *pde2* mutation does not confer a measurable increase in uptake. Our observation that mM concentrations of cAMP are required to raise internal concentrations attests to the inefficient uptake of cAMP in yeast.

3.4. *Elevated intracellular cAMP levels reduce thermotolerance*

We tested the effect of elevated intracellular cAMP levels on the thermotolerance of both log-phase and nutrient-arrested cells. Mid-log phase RW301 (*pde2::TRP1*) cells in MIN medium were shifted to 48°C and the loss in viability was determined over time (see section 2). The results are shown in Fig. 1. All but about 0.1% of the cells lost viability in the first 30 min after the shift to 48°C; the remaining cells were more thermotolerant and most remained viable after 240 min at 48°C (open circles). The addition of 4 mM cAMP to the medium (filled circles) did not dramatically affect the large fraction of thermosensitive cells, but the more thermotolerant fraction (again ~0.1%) lost viability more rapidly. The wild-type strain, RW300, showed a thermotolerance profile similar to RW301 in MIN medium irrespective of the presence of cAMP in the medium (data not shown).

Yeast cells starved for many essential nutrients acquire thermotolerance. To test the effect of elevated intracellular cAMP levels on the maintenance of thermotolerance of nutrient-arrested cells, we transferred mid-log phase cells to minimal, nitrogen-free medium (MIN−N) for 2 days, at which point greater than 80% of the cells remained viable after incubation at 48°C for 60 min. cAMP was then added to the arrested cells. After 6 h we shifted the cells to 48°C and determined viability loss over time (see section 2). RW300 (*PDE2*) cells remained completely viable after 60 min at 48°C, with or without exogenous cAMP (Fig. 2). In contrast, as cAMP was increased, the thermotolerance of RW301 (*pde2*) cells decreased (Fig. 2). After 6 h in 4 mM cAMP less than 5% of the cells remained viable at 48°C for 60 min. The fraction of unbudded cells, determined both before and after the addition of cAMP, was greater than 97%, indicating that increasing intracellular cAMP did not cause cells to re-enter the cell cycle.

Table II

Effects of increased intracellular cAMP levels on doubling time, the G_1 phase, and cell growth

Strain[a]	Medium[b]	Unbudded cells (%)	T_D[c] (h)	G_1[d] (h)	S + G_2 + M (h)	Diameter (μm)	Dry weight (mg/10^7 cells)	Protein (μg/10^7 cells)
RW300	MIN	55	3.50 ± 0.10	1.6	1.9	4.0	1.1 ± 0.1	46.1 ± 9.0
RW300	MIN + cAMP	51	3.48 ± 0.18	1.5	2.0	4.0	1.5 ± 0.2	52.8 ± 6.9
RW301	MIN	53	3.83 ± 0.37	1.7	2.1	4.2	2.5 ± 0.4	53.2 ± 5.1
RW301	MIN + cAMP	67	4.40 ± 0.03	2.6	1.8	4.7	5.8 ± 0.2	125.2 ± 19.3

[a] The RW300 (*PDE2*) and RW301 (*pde2*) cells were in mid-log phase.
[b] Exogenous cAMP was added, when indicated, to a concentration of 4 mM.
[c] T_D, doubling time; the 95% confidence intervals were derived from three separate experiments.
[d] The 95% confidence intervals are < 0.2 h in all cases.

4. DISCUSSION

Strains lacking the high-affinity cAMP phosphodiesterase behave normally under most laboratory conditions. Traits that are associated with very high intracellular cAMP levels, such as extreme heat-shock sensitivity, starvation intolerance and the inability to sporulate, are not observed for *pde2* or *rca1* mutants. These observations, as well as the fact that cAMP levels either do not increase or increase only slightly [2], indicates that this enzyme is not the unique component that buffers cAMP in cells. It has been reported that a strong feedback loop regulates cAMP production. The interruption of the feedback loop, such as that which occurs in cAPK mutants with low but constitutive levels of cAPK activity (*tpk*^{w} mutants), results in a several hundred-fold increase in cAMP levels irrespective of the presence of phosphodiesterase [5].

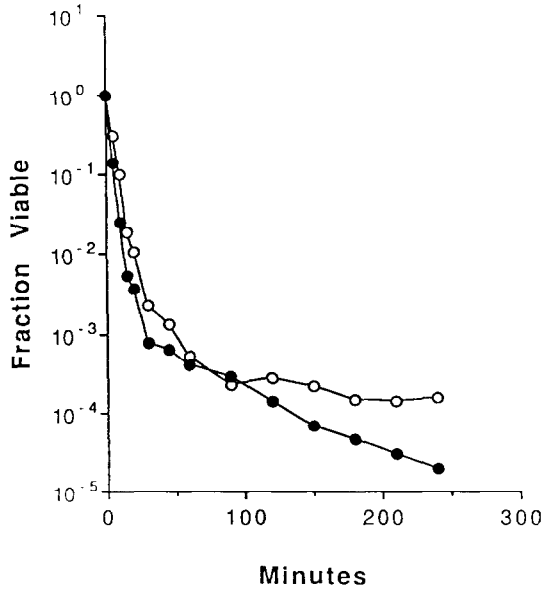

Fig. 1. Effect of elevated intracellular cAMP levels on the thermotolerance of exponentially growing cells. RW301 (*pde2*) cells in mid-log phase were shifted to 48°C and removed to determine viability at the times indicated (see section 2). The 95% confidence intervals were in most cases approximately the size of the plot symbols, each of which represents the average of two repetitions of the experiment. ○, MIN medium; ●, MIN+4 mM cAMP.

Exogenous cAMP is able to activate most of the cAMP-dependent events in *pde2/rca1* cells, in contrast to wild-type cells which are insensitive to cAMP. The addition of cAMP bypasses the essential requirement for *CDC25*, *RAS1* and *RAS2*, and adenylyl cyclase (*CDC35/CYR1*) and induces physiological changes similar to those found in strains with hyperactive cAPK. Addition of cAMP to the medium causes *pde2* mutant strains to lose viability when starved for nitrogen and, as reported here, causes nutritionally arrested cells to lose thermotolerance. These results imply that cAMP increases within the cell. This phenotype was observed independently for *rca1* mutants and was attributed to changes in permeability of the cell to cAMP [8]. From genetic crosses presented above it is clear that *rca1* and *pde2* are allelic, therefore the internal increase of cAMP has to be re-interpreted. The measurement of cAMP in the *pde2* deletion strain shows a real increase after exogenous cAMP addition despite the fact that an increase in direct uptake could not be detected. The simplest explanation is to assume that the yeast membrane is slightly permeable to cAMP; when the cell is challenged to high concentrations, the permeability barrier is insufficient to prevent leakage sufficient to activate the cAPK.

The *pde2/rca1* mutations thus allow the use of cAMP as a pharmacological agent to manipulate endogenous cAMP levels from the outside without temperature shifts which are known to affect the stress response, thermotolerance, sporulation, and carbohydrate accumulation. Bissinger et al. [20] and Baroni et al. [21] have used the *rca1* mutation to demonstrate cAMP-dependent control of catalase T transcription and the cell cycle, respectively. François et al. [22] used the *pde2* mutation to investigate the role of cAMP on trehalose biosynthesis. The *pde2* mutation also has an advantage over the use of the polygenic *cam* genotype [19], because a *pde2* null allele can be introduced directly into any strain background by transformation. Using a *pde2* strain, we

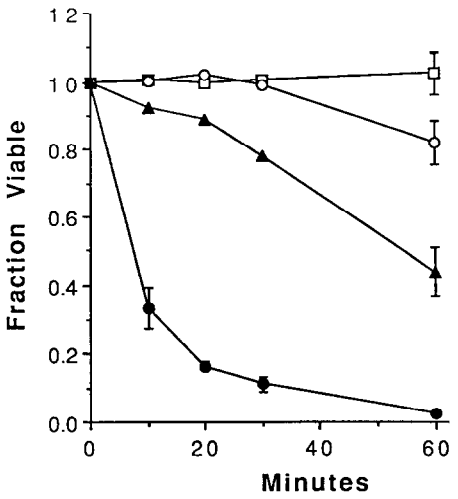

Fig. 2 Effects of elevated intracellular cAMP levels on the thermotolerance of nutrient-arrested cells. Exponentially growing RW300 (*PDE2*) and RW301 (*pde2*) cells in mid-log phase were arrested for 2 days in MIN−N medium, transferred to MIN−N medium plus exogenous cAMP for 6 h, and then shifted to 48°C and removed to determine viability at the times indicated (see section 2). □, RW300 (*PDE2*) in MIN−N+4 mM cAMP; ○, RW301 (*pde2*) in MIN−N; ■, RW301 (*pde2*) in MIN−N+2 mM cAMP; ●, RW301 (*pde2*) in MIN−N+4 mM cAMP.

raised intracellular cAMP levels in cells previously arrested by starvation and found that, as with an *rca1* strain [8], thermotolerance was rapidly lost. The acquisition of thermotolerance has been hypothesized to be characteristic of yeast cells in the G_0 state [23–25]. If this is true, then our results demonstrate that low intracellular cAMP is required to maintain the G_0 state.

In spite of a very poor permeability to cAMP, at very high external cAMP concentrations the high-affinity phosphodiesterase may play a protective role. Based on similar evidence, such a role for the cAMP phosphodiesterase in coliform bacteria has been hypothesized [26,27]. A wide variety of microorganisms, ranging from coliform bacteria [28,29] to *Dictyostelium* [30] and yeast [31], secrete cAMP. In the case of *E. coli*, greater than 99% of the cAMP synthesized is found in the culture medium [28]. We hypothesize that *S. cerevisiae*, to avoid the deleterious effects of extracellular cAMP, has developed a safety system composed of a membrane barrier, active extrusion, and the high-affinity phosphodiesterase.

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REFERENCES

[1] Broach, J.R. and Deschenes, R.J (1990) Adv Cancer Res. 54, 79–139.
[2] Nikawa, J., Sass, P and Wigler, M. (1987) Mol. Cell. Biol. 7, 3629–3636
[3] Sass, P., Field, J., Nikawa, J , Toda, T. and Wigler, M. (1986) Proc. Natl. Acad. Sci. USA 83, 9303–9307.
[4] Wilson, R.B. and Tatchell, K. (1988) Mol Cell. Biol. 8, 505–510.
[5] Nikawa, J I , Cameron, S , Toda, T., Ferguson, K.M. and Wigler, M. (1987) Genes Dev. 1, 931–937.
[6] Cobitz, A.R., Yim, E.H., Brown, W.R , Perou, C.M. and Tamanoi, F. (1989) Proc. Natl Acad. Sci USA 86, 858–862.
[7] Gross, E., Goldberg, D. and Levitzki, A. (1992) Nature 360, 762–764.
[8] Boy-Marcotte, E., Garreau, H and Jacquet, M. (1987) Yeast 3, 85–93.
[9] Jacquet, M., Camonis, J., Boy-Marcotte, E., Damak, F. and Garreau, H (1989) in: The Guanine-nucleotide Binding Proteins (L Bosch, B. Kraal, and A. Parmeggiani eds.) Plenum Press, NY, pp. 241–249.
[10] Johnston, G.C., Pringle, J.R. and Hartwell, L.H. (1977) Exp. Cell. Res 105, 79–98
[11] Maniatis, T., Sambrook, J. and Fritsch, E.F., (1982) in: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[12] Ito, H., Fukuda, Y., Murata, K. and Kimura, A. (1983) J. Bacteriol 153, 163–168.
[13] Winston, F., Chumley, F. and Fink, G.R. (1983) Methods Enzymol. 101, 211–228.
[14] Garbers, D L., Tubb, D.J. and Hyne, R.V. (1982) J. Biol Chem. 275, 8980–8984.
[15] Jacobs, K.H., Bohme, E. and Schultz, G. (1976) in: Regulation of Function and Growth of Eukaryotic Cells by Intracelluar Cyclic Nucleotides (Dumont, J.E., Brown, B.L. and Marshall, N.J eds) Plenum Press, NY, pp. 295–311.
[16] Harper, J.F. and Brooker, G. (1975) J. Cycl. Nucl. 1, 207–218.
[17] Bradford, M.M (1976) Anal. Biochem. 72, 248–254.
[18] Rivin, C J. and Fangman, W.L (1980) J Cell Biol. 85, 96–107.
[19] Matsumoto, K., Uno, I , Toh-e, A , Ishikawa, T and Oshima, Y. (1982) J. Bacteriol. 150, 277–285.
[20] Bissinger, P.H , Wieser, R., Hamilton, B and Ruis, H (1989) Mol. Cell Biol. 9, 1309–1315
[21] Baroni, M.D., Monti, P., Marconi, G. and Alberghina, L (1992) Exp Cell Res. 201, 299–306.
[22] François, J., Neves, M.-J. and Hers, H.-G. (1991) Yeast 7, 575 587.
[23] Iida, H and Yahara, I. (1984) J. Cell. Biol. 98, 1185–1193.
[24] Iida, H and Yahara, I. (1984) J Cell. Biol. 99, 199–207
[25] Plesset, J., Ludwig, J.R , Cox, B.S and McLaughlin, C S. (1987) J Bact 169, 779–784.
[26] Alper, M.D. and Ames, B.N. (1975) J. Bacteriol. 122, 1081–1090.
[27] Botsford, J.L. (1984) J. Bacteriol. 160, 826–830.
[28] Matin, A and Matin, M.K (1982) J Bacteriol 149, 801–807
[29] Wright, L.F., Milne, D.P. and Knowles, C.J. (1979) Biochim. Biophys. Acta 583, 73–80.
[30] Bonner, J.T., Barkley, D S., Hall, E.M., Konijn, T.M., Mason, J.W., O'Keefe, G. and Wolfe, P.B. (1969) J. Bacteriol. 102, 682–687.
[31] Smith, M.E , Dickinson, J.R. and Wheals, A.E. (1990) Yeast 6, 53–60.