Glucose exerts opposite effects on mRNA versus protein and activity levels of Pde1, the low-affinity cAMP phosphodiesterase from budding yeast, *Saccharomyces cerevisiae*

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Abstract In budding yeast (*Saccharomyces cerevisiae*), a low-affinity phosphodiesterase, Pde1, and a high-affinity phosphodiesterase, Pde2, are responsible for the degradation of cAMP. Addition of glucose to glycerol-grown yeast cells is known to cause a transient increase in the cAMP level and recent work has indicated a specific involvement of Pde1 in this response. In this work we show that glucose addition induces the accumulation to high levels of mRNA encoding Pde1. This increase continues for at least 8 hours and is due to enhanced transcription of the *PDE1* gene, since glucose addition does not change the stability of the Pde1 mRNA. Surprisingly, using an assay method specific for Pde1, we observed that the activity of Pde1 remains constant and finally decreases several-fold during the same period. In addition, this activity profile closely follows the Pde1 protein level as judged from Western blotting with antibodies directed against Pde1. Experiments using cycloheximide, a general inhibitor of translation, allow to exclude the possibility of a futile cycle of Pde1 synthesis and degradation. Hence, glucose addition appears to trigger an increase in *PDE1* gene transcription together with a specific inhibition of the translation of Pde1 mRNA.

Key words: Phosphodiesterase; cAMP; Glucose-induced signalling; *Saccharomyces cerevisiae*

1. Introduction

Saccharomyces cerevisiae phosphodiesterase 1 (Pde1) catalyzes the degradation of cAMP with a K_m between 20 μM and 250 μM depending on the conditions [1,2]. This low-affinity phosphodiesterase is unrelated in amino-acid sequence to the second phosphodiesterase from budding yeast, Pde2, which displays a much lower K_m of 170 nM [3]. Whereas Pde2 is a member of a large family of phosphodiesterases with homologs in several species [4], database searching using the basic local alignment search tool (BLAST) program [5] revealed significant ($P < 0.05$) homology of Pde1 with only four other phosphodiesterases from both eukaryotic (*Dictyostelium discoideum*, *Candida albicans* and *Schizosaccharomyces pombe*) and prokaryotic (*Vibrio fischeri*) origin. The best conserved region is shown in Fig. 1. Until now no homologs have been found in higher eukaryotes indicating that the enzyme may have been lost during evolution, and its function taken over by another class of phosphodiesterases or that it has remained undetected. Alternatively, Pde1 may fulfil a specific function which is not required in higher eukaryotes. In this respect it is noteworthy that in *D. discoideum* this phosphodiesterase functions to maintain the responsiveness to the chemoattractant cAMP during the aggregation phase of development [6], whereas it confers the unusual property of growth on cAMP to the marine bacterium *V. fischeri* [7]. *PDE1* is not an essential gene for *S. cerevisiae* and its deletion has only minor effects on the basal cAMP levels and the phenotypic characteristics controlled by cAMP-dependent protein kinase [8]. However it has been suggested that it might play an important role in the degradation of the high cAMP concentrations that occur transiently in yeast after glucose addition [2] and recent work has supported this proposal (Ma et al., in preparation). cAMP levels from yeast cells grown on glucose for a longer time (glucose-repressed cells) no longer respond to glucose addition [9].

As a further step towards the elucidation of the function of Pde1 in budding yeast, we studied the expression and the activity of this enzyme as a function of time after glucose addition. Addition of glucose to *S. cerevisiae* cells grown on a non-fermentable carbon source is known to influence the expression of many genes by means of different signalling pathways and also results in rapid posttranslational changes in enzyme activity triggered by various signalling pathways [10,11].

We noticed that after glucose addition Pde1 activity and protein levels initially remained unchanged and afterwards clearly decreased, suggesting that also the expression of *PDE1* might be downregulated during growth on glucose. Unexpectedly, however, after glucose addition the mRNA levels of Pde1 continued to increase for several hours, suggesting the existence of novel mechanisms, at the level of translation, by which yeast cells adapt to growth on glucose.

2. Material and methods

2.1. Yeast strains and glucose-induction experiments

The following strains were used: wild-type strain SP1 (*Mat**a** leu2*

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C. albicans (113-140)     NNLQNMLISHSHLDHVCSVVINSAGFNK
S. pombe (105-132)        QRIKTFLITHCHLDHIYGAVINSAMFGP
S. cerevisiae (119-146)   QGLTDYYITHFHLDHISGLVVNSPSIYE
D. discoideum (161-188)   KHVMSYFIGHSHLDHVGGLILVSPEDYL
V. fischeri (95-122)      DRIKGYFISHAHLDHVAGLIISSPDDSK
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Fig. 1. Multiple alignment of a conserved region of the Pde1 homologs from *Saccharomyces cerevisiae* (P22434), *Candida albicans* (P32782), *Schizosaccharomyces pombe* (P36599), *Dictyostelium discoideum* (A25346) and *Vibrio fischeri* (A40602). The numbers between brackets refer to the displayed amino-acid residues. Residues that are identical in at least three of the five proteins are boxed.

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate

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his3 trp1 ade8 can1 ura3), *pde1 pde2* double deletion strain DJ23-3C (*Matα his3 leu2 ura3 trp1 ade8 pde1::LEU2 pde2::URA3*), *pde1* deletion strain J105 (*Mat**a** leu2 his3 ura3 trp1 ade8 can1 pde1::LEU2*) and *pde2* deletion strain J104 (*Mat**a** leu2 his3 ura3 trp1 ade8 can1 pde2::HIS3*). All strains were grown in YP medium (2% (w/v) bactopeptone, 1% (w/v) yeast extract) containing either 2% (w/v) glucose or 2% (v/v) glycerol. Cycloheximide (Sigma) was used at a concentration of 10 mg/l and added 20 min before the start of the experiment.

For glucose-induction experiments, yeasts were grown in medium containing 2% glycerol until $OD_{600} = 1$. Glucose was added to a final concentration of 2% and aliquots were taken before glucose addition and at the indicated time points. Ten hours after glucose addition (in the absence of cycloheximide) cultures reached an OD_{600} of 6.1–6.7.

2.2. Phosphodiesterase assay

Phosphodiesterase activity was assayed at 30°C by measuring the time-dependent degradation of cAMP in a mixture containing assay buffer (50 mM Tris-HCl pH 8, 0.1 mM EDTA), 500 μM cAMP and the indicated amounts of protein. The reaction was stopped by diluting aliquots of the reaction mix 200-fold in water and heating of the diluted sample. cAMP was assayed subsequently, using the cAMP [^{3}H] assay system from Amersham.

Yeast cells were harvested during the exponential phase of growth by centrifugation, washed once in cold assay buffer and recentrifuged. Yeast extracts were prepared by breaking the yeast cells with glass beads in assay buffer containing 0.2 mM phenylmethanesulfonyl fluoride, followed by centrifugation of the homogenates at high speed (10 min at $20000 \times g$). Protein concentration was determined by the Lowry method [12].

2.3. Western blotting

Extracts were boiled in sample buffer and applied on 15% SDS-PAGE. Following electrophoresis, gels were blotted on nitrocellulose membranes (Hybond C, Amersham), blocked with 5% skimmed milk and 0,2% Tween-20 in Tris-buffered saline and developed with primary and secondary (alkaline phosphatase conjugated anti-rabbit monoclonal antibody from Sigma Immunochemicals) antisera. The secondary antibody was visualised with 4-nitro blue tetrazolium (Boehringer Mannheim) and 5-bromo-4-chloro-3-indolyl-phosphate (Boehringer Mannheim) at alkaline pH.

The primary antiserum, directed against the synthetic peptide CKSTPAKRDPRLTILE (corresponding to residues 328–342 of Pde1 plus an additional N-terminal cysteine), was raised in a rabbit (Eurogentec, Liège, Belgium) and used in a 1/50 dilution to detect Pde1 on Western blots. The specificity of the signal was confirmed by comparing samples from wild-type and *pde1* deletion strains developed with either antiserum or pre-immune serum.

2.4. Northern blotting and RNA stability experiments

RNA was prepared and Northern blotting was performed as described previously [13,14]. Blots were developed with a probe corresponding to a *Rsa*I fragment (nucleotides 134–1113 of the coding region) of *PDE1*. Equal loading of the samples was verified by developing the blots with a probe specific for actin (the 3.25 kb *Eco*RI-*Bam*HI restriction fragment from pYA301) [15].

To measure the stability of the Pde1 mRNA, the coding region and the promoter of the *PDE1* gene were cloned in a YEplac195 vector using the *Xba*I and *Sma*I sites. Wild-type yeast cells were transformed with this vector and grown on ura$^-$ synthetic medium containing 2% (v/v) glycerol. When grown on glycerol these transformed cells accumulate Pde1 mRNA in response to glucose addition in a similar way as wild-type cells. Hundred μg/ml of the transcriptional inhibitor 1,10-phenanthroline [16] was added either alone or in the presence of 2% glucose and samples were taken at several time points and processed for Northern blotting with probes specific for either actin or Pde1.

3. Results and discussion

3.1. Addition of glucose to glycerol-grown cells induces the accumulation of Pde1 mRNA

Wild-type yeast cells were grown in glycerol-containing medium to an optical density of 1. To investigate the effect of glucose on Pde1 expression, glucose was added to a final concentration of 2% (w/v) and samples were taken at several time points. Total RNA was prepared and equal amounts were analysed by Northern blotting. As shown in Fig. 2A, hybridisation of the blot with a probe specific for *PDE1*, revealed that the concentration of this messenger increased several-fold after the addition of glucose over a time period of 8 h. As shown in Fig. 2B, hybridisation of the blot with an actin-specific probe revealed that this increase was specific for Pde1 and not due to a general activation of transcription upon glucose addition.

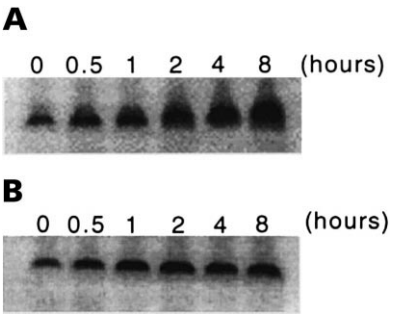

Fig. 2. Addition of glucose to glycerol-grown cells results in the accumulation of Pde1 mRNA. Wild-type yeast cells were grown in glycerol-containing medium to $OD_{600} = 1$. 2% (w/v) glucose was added and aliquots were taken before and at the indicated time points after addition of glucose. Total RNA was prepared and analysed by Northern blotting as described in Section 2. Blots were hybridised with either a *PDE1*-specific probe (A) or an actin probe (B).

We also tested the effect of glucose on the Pde1 mRNA level in a strain carrying a deletion of the *PDE2* gene, that encodes the high-affinity phosphodiesterase. In such cells Pde1 is solely responsible for cAMP degradation. These *pde2* cells responded in a very similar way to glucose addition as wild-type cells in terms of the accumulation of Pde1 mRNA (not shown).

Since the Pde1 phosphodiesterase appears to play a specific role in the degradation of the transient cAMP spike upon addition of glucose to derepressed cells and since glucose-repressed cells no longer respond to glucose addition with a cAMP increase, the strong continued increase in Pde1 mRNA after glucose addition was rather unexpected.

3.2. A specific assay method for Pde1

Phosphodiesterase activity can be assayed by measuring the time and dose-dependent degradation of exogenous cAMP added to yeast cell extracts. Normally, both phosphodiesterases, Pde1 and Pde2, will contribute to this degradation. To develop an assay specific for the low-affinity phosphodiesterase Pde1, we took advantage of the extensive knowledge [1–3,17] of the enzymatic properties of Pde1 and Pde2. Firstly, the high K_m of Pde1 allows an assay at very high cAMP concentrations. Secondly, Pde1 activity is independent of divalent cations, which are, however, indispensable for the activity of Pde2. We reasoned that in the absence of divalent cations (0.1 mM EDTA) and in the presence of a high (500 μM) cAMP concentration, the degradation of cAMP would reflect the activity of Pde1. Using this assay method we found indeed equal phosphodiesterase activities in extracts from wild-type and *pde2* strains, but no activity in a *pde1* nor in a *pde1 pde2* double deletion strain (Fig. 3). These data confirm the specificity of our assay method for Pde1.

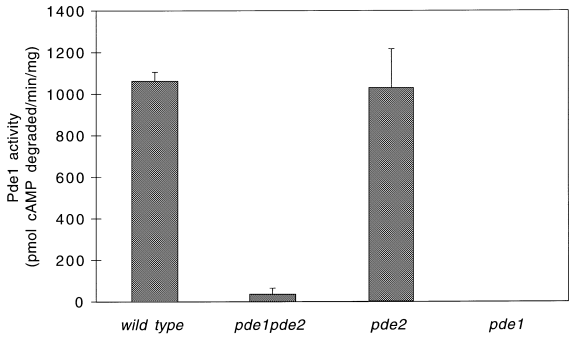

Fig. 3. Specificity of the assay method for Pde1. Wild-type yeast cells and *pde1 pde2*, *pde1* and *pde2* strains were grown in YP medium containing 2% (w/v) glucose to $OD_{600} = 1$. Extracts were prepared and phosphodiesterase activity was assayed as described in Section 2. Results are expressed as means ± S.E.M. of three experiments.

3.3. *Pde1-specific activity and Pde1 protein levels remain constant and finally decrease after the addition of glucose to glycerol-grown cells*

This Pde1 assay was subsequently used to investigate Pde1 activity following addition of glucose to glycerol-grown cells. Although glucose induces a remarkable increase in the concentration of Pde1 mRNA (Fig. 2), Pde1-specific activity remained constant during the first 4 h after which a pronounced decrease was observed (Fig. 4A). Ten h after the addition of glucose the Pde1 activity had dropped to about 20% of the original value. Similar results were obtained when a *pde2* strain was used (not shown).

These observations could either be explained by a post-translational modification that switches off the enzyme so that in spite of higher or equal Pde1 concentrations, Pde1 activity decreases, or by a decrease in the Pde1 protein concentration itself. This question was investigated using antibodies directed against Pde1. Total cell extracts were prepared from samples, taken at several time points after addition of glucose, and analysed by Western blotting. As shown in Fig. 4B, the Pde1 concentration initially remained constant and decreased after 8 h. Hence, under these conditions, Pde1 activity reflects the Pde1 concentration. This means that although glucose causes an increase in Pde1 mRNA concentration, the actual Pde1 protein concentration remains unchanged and at a later stage decreases significantly. Taken together, these data can only be explained when glucose exerts a translational inhibition on the Pde1 messenger and/or promotes the degradation of the Pde1 protein.

To distinguish between these two possible explanations we measured the effect of glucose addition to yeast cells in the presence of a general inhibitor of translation. If glucose induces a futile cycle of Pde1 synthesis and degradation, blocking protein synthesis with cycloheximide should result in a rapid glucose-induced degradation of Pde1. Fig. 5 shows, however, that also in the presence of cycloheximide Pde1 levels remain constant during the first 4 h following glucose addition. Moreover cycloheximide prevents the degradation of Pde1 that occurs in non-treated cells 8 h after glucose addition, indicating that this late response requires protein synthesis. Since Pde1 synthesis is not required for the maintenance of a constant Pde1 concentration following glucose addition, a translational control mechanism, rather than a futile cycle of Pde1 synthesis and degradation, operates under these conditions. Such a glucose-induced inhibition of the translation of specific messengers might represent a novel mechanism by which yeast cells adapt to growth on glucose. The same mechanism might also explain the opposite behaviour of the Tps1 messenger and protein previously reported [14]. From our results, it is also clear that the observation of

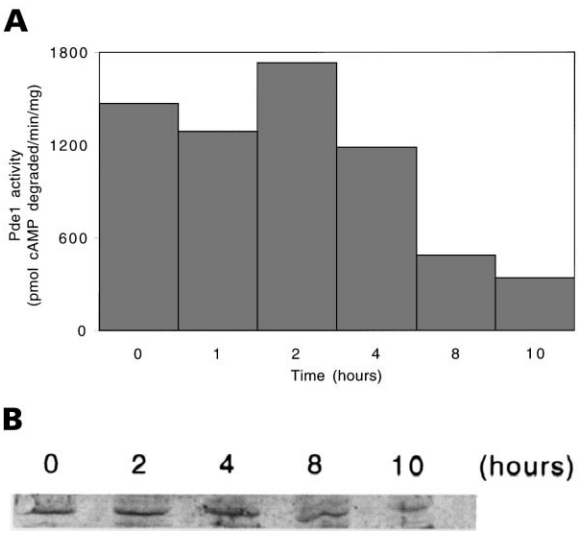

Fig. 4. Addition of glucose to glycerol-grown cells leads to a decrease in Pde1-specific activity and Pde1 concentration. Wild-type yeast cells were grown in glycerol-containing medium. Glucose was added to a final concentration of 2% and aliquots were taken before and at the indicated time points after addition of glucose. A: Extracts were prepared and assayed for Pde1 activity as described in Section 2. Data from a typical experiment are illustrated. B: Extracts were prepared and the amount corresponding to 10 μg protein was boiled in sample buffer and analysed by Western blotting with a Pde1-specific antibody.

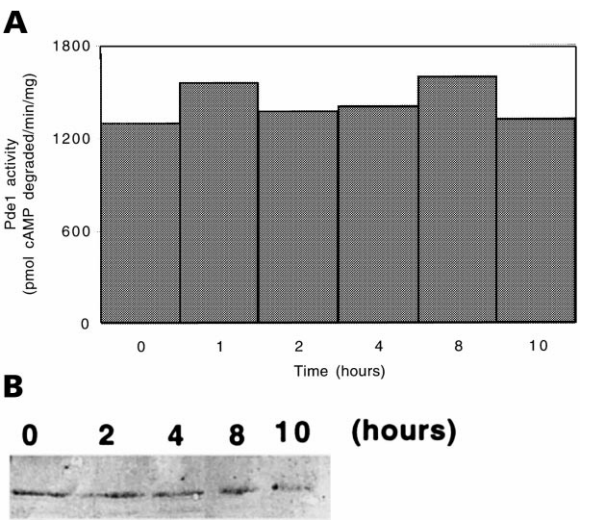

Fig. 5. Effect of glucose addition on Pde1 activity in the presence of cycloheximide. Wild-type yeast cells were grown in glycerol-containing medium and cycloheximide was added 20 min before the start of the experiment. Glucose was added to a final concentration of 2% and aliquots were taken before and at the indicated time points after addition of glucose. A: Extracts were prepared and assayed for Pde1 activity as described in Section 2. Data from a typical experiment are illustrated. B: Extracts were prepared and the amount corresponding to 10 μg protein was boiled in sample buffer and analysed by Western blotting with a Pde1-specific antibody.

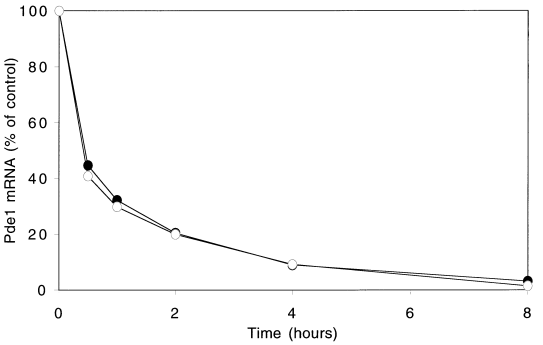

Fig. 6. Glucose does not affect the stability of the Pde1 mRNA. The stability of Pde1 mRNA was determined as described in Section 2. Yeast cells were grown in glycerol-containing medium. At time point 0 phenanthroline was added either alone (○) or in the presence of 2% (w/v) glucose (●). Samples were taken at the indicated time points and analysed by Northern blotting with a *PDE1*-specific probe and quantitated using phosphorimager technology. Data from a typical experiment are illustrated.

alterations in the concentration of a given mRNA is not sufficient to conclude that corresponding changes in the enzymatic activity or protein level occur.

3.4. Glucose does not change the stability of the Pde1 mRNA

Glucose is known to alter the expression of several genes in budding yeast [10,11], and this effect might account for the rise in Pde1 mRNA concentrations described here. On the other hand, glucose triggers a specific inhibition of the translation of the Pde1 messenger which might lead to a stabilisation of this messenger since most mRNAs need to be translated in order to be degraded [18]. To distinguish between these two possibilities we checked whether glucose addition to cells grown on glycerol changed the half-life of the Pde1 mRNA. For this purpose transcription was blocked by the addition of 1,10-phenanthrolin and the subsequent disappearance of Pde1 mRNA was followed by Northern blotting (Fig. 6). In cells grown on glycerol the half-life of Pde1 mRNA was 25.5 min and the mRNA disappeared almost completely ($< 5\%$ of the initial amount) after 8 h. Addition of 2% glucose had no significant effect on these kinetics (half-life of 22.5 min, complete disappearance after 8 h). Since glucose addition did not stabilise the Pde1 messenger we can conclude that the observed accumulation of Pde1 mRNA is caused by enhanced transcription of the *PDE1* gene.

3.5. Conclusions

The picture that emerges is thus that glucose addition to yeast cells grown on a non-fermentable carbon source leads to enhanced transcription of the *PDE1* gene and to accumulation of Pde1 mRNA. Due to a translational control system the amount of Pde1 protein and Pde1 activity remain constant and finally decrease. At present we can only speculate on the reasons why the cells accumulate Pde1 mRNA which is not translated. One possibility is that this mechanism allows a rapid resynthesis of Pde1 once glucose is exhausted.

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