Involvement of the $CDC25$ gene product in the signal transmission pathway of the glucose-induced RAS-mediated cAMP signal in the yeast *Saccharomyces cerevisiae*

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Addition of glucose or related fermentable sugars to derepressed cells of the yeast *Saccharomyces cerevisiae* triggers a RAS-protein-mediated cAMP signal, which induces a protein phosphorylation cascade. Yeast strains without a functional $CDC25$ gene were deficient in basal cAMP synthesis and in the glucose-induced cAMP signal. Addition of dinitrophenol, which in wild-type strains strongly stimulates in *vivo* cAMP synthesis by lowering intracellular pH, did not enhance the cAMP level. $cdc25$ disruption mutants, in which the basal cAMP level was restored by the $RAS2^{val19}$ oncogene or by disruption of the gene ($PDE2$) coding for the high-affinity phosphodiesterase, were still deficient in the glucose- and acidification-induced cAMP responses. These results indicate that the $CDC25$ gene product is required not only for basal cAMP synthesis in yeast but also for specific activation of cAMP synthesis by the signal transmission pathway leading from glucose to adenyl cyclase. They also show that intracellular acidification stimulates the pathway at or upstream of the $CDC25$ protein. When shifted to the restrictive temperature, cells with the temperature sensitive $cdc25-5$ mutation lost their cAMP content within a few minutes. After prolonged incubation at the restrictive temperature, cells with this mutation, and also those with the temperature sensitive $cdc25-1$ mutation, arrested at the 'start' point (in $G_1$) of the cell cycle, and subsequently accumulated in the resting state $G_0$. In contrast with $cdc25-5$ cells, however, the cAMP level did not decrease and normal glucose- and acidification-induced cAMP responses were observed when $cdc25-1$ cells were shifted to the restrictive temperature. These results show that, in the original genetic background at least, growth arrest of $cdc25-1$-bearing cells at the restrictive temperature is not due to cAMP deficiency. Previous experiments have provided evidence for the presence of a glucose-repressible protein in the signalling pathway. Exponential-phase glucose-grown cells of a strain with overexpression of $CDC25$ unexpectedly showed a glucose-induced cAMP signal. Control experiments, however, indicated that overexpression of $CDC25$ caused a defect in glucose repression. Introduction of the $cat1$ derepression mutation in the strain with overexpression of $CDC25$ restored glucose repression and abolished the glucose-induced cAMP signal, indicating that the $CDC25$ protein is not the glucose-repressible component of the signalling pathway.

**Introduction**

When glucose or related fermentable carbon sources are added to derepressed cells, stationary-phase cells or to ascospores of the yeast *Saccharomyces cerevisiae*, a cAMP signal is induced which triggers a protein phosphorylation cascade, similar to the hormone-induced cascades in mammalian cells (for a recent review, see Thevelein, 1988). The mechanism by which glucose triggers the cAMP signal is not well understood. It has been shown that transient plasma membrane depolarization, transient intracellular acidification and increased energy supply are not triggers for the cAMP signal. Recent studies have provided evidence for the existence of a specific signal transmission pathway. The RAS proteins, which in yeast probably act in a way similar to the G$_s$ proteins of mammalian adenyl cyclase (Defeo-Jones et al., 1983, 1985; Dhar et al., 1984; Kataoka et al., 1984, 1985; Toda et al., 1985), have been implicated as signal transmitters in the pathway (Mbonyi et al., 1988). The requirement of glucose metabolism for induction of the cAMP signal is very limited. Transport of glucose alone is unable to induce the cAMP signal, but the presence of a functional sugar kinase (hexokinase 1, hexokinase 2 or...
glucokinase) in the only other requirement. Whether the kinase is needed for phosphorylation of the sugar, or for another reason, is not yet clear (Beullens et al., 1988). Experiments with a mutant lacking the SNF3 gene, which encodes the high-affinity glucose carrier, have shown that this carrier is not required for induction of the cAMP signal (Mbonyi & Thevelein, 1988). This implies that the putative mechanism of transport-associated phosphorylation of sugar is not involved in induction of the cAMP signal (Beullens & Thevelein, 1990).

The yeast CDC25 protein forms a functional complex with the RAS–adenyl cyclase system (Broek et al., 1987; Camonis et al., 1986; Daniel, 1986; Daniel et al., 1987; Martegani et al., 1986a; Robinson et al., 1987). In the absence of CDC25 or RAS proteins, the basal cAMP level in yeast cells is extremely low (Broek et al., 1987). To differentiate between the alternatives that the CDC25 gene product is merely needed to sustain basal cAMP synthesis and that it acts as a component of the signal transmission pathway leading from glucose to adenyl cyclase, we have investigated glucose-induced cAMP signalling in cdc25 mutants in which the capacity for basal cAMP synthesis was restored by the presence of the RAS2val19 oncogene in the genome or on a plasmid. These strains always contained at least one additional wild-type RAS gene which allows for transmission of the glucose-induced cAMP signal, even in the presence of the RAS2val19 oncogene product (Mbonyi et al., 1988). These experiments showed that the CDC25 protein functions as a component of the signalling pathway. Recent experiments by Munder & Küntzel (1989) also pointed to involvement of CDC25 in glucose-induced cAMP signalling.

In vivo cAMP synthesis in yeast is strongly stimulated by intracellular acidification (Caspari et al., 1985; Purwin et al., 1986; Thevelein et al., 1987a). Previous evidence indicated that lowered intracellular pH does not act directly on adenyl cyclase, but rather on the RAS proteins or on other controlling elements situated upstream in the activation pathway of the enzyme (Mbonyi et al., 1988). In this paper, we have investigated whether a functional CDC25 protein is required for the acidification-induced increase in cAMP. Experiments with glucose-repressed and derepressed wild-type cells, and with repression and derepression mutants, have shown that fermentable sugar is able to induce a cAMP signal only in derepressed cells and not in glucose-repressed cells. This has led to the hypothesis that the signalling pathway contains a glucose-repressible protein (Beullens et al., 1988; Argüelles et al., 1990; Mbonyi et al., 1990). We have made use of strains with overexpression of CDC25 to determine whether CDC25 is the putative glucose-repressible protein of the pathway.

Temperature-sensitive cdc25 mutants behave as cell cycle mutants. They arrest at the restrictive temperature at a point called 'start' in the G1 phase of the cell cycle and subsequently accumulate as round unbudded cells in the resting state G0. The same is true for temperature-sensitive mutants in CDC35, the gene for adenyl cyclase (Boutelet et al., 1985; Casperson et al., 1985; Kataoka et al., 1985). Whereas cdc25 disruption mutants clearly have very low cAMP levels (Broek et al., 1987), conflicting results have been published for cAMP levels in cells bearing temperature-sensitive alleles of CDC25, incubated at restrictive temperatures. Experiments with cells carrying the cdc25-5 allele showed a rapid drop of the cAMP level upon shift to the restrictive temperature (Camonis et al., 1986). On the other hand, in cells of the cdc25-1 mutant the cAMP level did not significantly change (Martegani et al., 1986b; Portillo & Mazon, 1986). These results were obtained by different authors using different methods for cAMP extraction and determination. Because of the proposed importance of the CDC25/RAS/adenyl cyclase pathway for progression over the 'start' point of the yeast cell cycle we have reinvestigated the possible difference between cdc25-1 and cdc25-5 and found it, at least in the original genetic background of the two strains, to be true.

Methods

Yeast strains. Saccharomyces cerevisiae strains used in this study are shown in Table 1. Plasmid pl.25S-P [a derivative of YRP7 (TRP1) which contains the CDC25 gene] was kindly provided by M. Jacquet (Orsay, France) and introduced into strains SP1, JT2000 and JT210 to give, respectively, strains JT4101, JT4200 and JT4300 (Table 1). Plasmid YE3 (RAS2val19 + LEU2) was isolated from strain TTA3-A, kindly provided by M. Wigler (Cold Spring Harbor) and introduced into strain SP1 to give strain JT4100. Strain JT1832 was obtained from a cross between strain TK161-R2V and strain TT1A-2 after selection of the diploid for plasmid (LEU2) loss. Heat shock sensitivity, basal trehalase activity and trehalose levels in the newly constructed strains were consistent with previous results reported in the literature (Broek et al., 1987). Strain JT5011 (cdc25-pde2) was obtained from a cross between strains JT5001 (pde2) and T139-5A-6A (cdc25::HIS3) + YE3 (RAS2val19) after selection of the diploid for plasmid (LEU2) loss. Strains JT2000 (cat) and JT210 (CAT1) were obtained from a cross between HRX.42-13C (cat1), kindly provided by K-D. Entian (Tübingen, FRG) and DBY747 (wild-type).

Culture and heat shock conditions. The cells were grown to a density of about 8 mg (wet weight) ml⁻¹ in a gyrotary incubator (200 r.p.m.) at 22 °C (temperature-sensitive strains), 37 °C (temperature-sensitive strains containing plasmids) or 30 °C (all other strains). Composition of the growth media was as follows. YPG: 3% (w/v) glycerol, 2% (w/v) peptone (Merck 7224) and 1% (w/v) yeast extract.YPD: 2% (w/v) glucose, 2% (w/v) peptone and 1% (w/v) yeast extract. YPGal: 2% (w/v) galactose, 2% (w/v) peptone and 1% (w/v) yeast extract. Strains in which the plasmid is not required for growth on rich media were cultured on minimal media (Sherman et al., 1985) lacking the nutrient specified by the selectable marker on the plasmid.

Heat shock was performed by replica-plating cell patches to plates (YPD or minimal glucose medium without leucine) that had been preheated at 55 °C. The plates were incubated for 30, 60, 90 or 120 min.
Glucose-induced cAMP signal in yeast

Results and Discussion

Stimulation of cAMP synthesis by glucose and by intracellular acidification

Addition of 100 mM-glucose or 2 mM-dinitrophenol (DNP) to wild-type yeast cells grown on non-fermentable carbon sources triggers a rapid increase in the cAMP level (Fig. 1). With glucose, the cAMP increase is always transient so that it takes the form of a cAMP signal. A yeast strain with a disrupted CDC25 gene in which viability was restored by a multicopy plasmid carrying the TPK1 gene has a very low cAMP level (Broek et al., 1987) and did not show a glucose- or DNP-induced cAMP increase (results not shown). To investigate whether the CDC25 protein truly acts as a component of the signalling pathway, or whether it is merely required for the viability, or whether the CDC25 protein truly acts as a component of the signalling pathway, or whether it is merely required

Table 1. Saccharomyces cerevisiae strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source (and reference)</th>
</tr>
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<tr>
<td>A364A</td>
<td>MATa ade 1 ade 2 ura 1 his 7 lys 2 tyr 1 gal 1</td>
<td>Yeast Genetic Stock Center</td>
</tr>
<tr>
<td>SP1</td>
<td>MATa his 3 leu 2 ura 3 trp 1 ade 8 can 1</td>
<td>M. Wigler (Toda et al., 1985)</td>
</tr>
<tr>
<td>TK161-R2V</td>
<td>MATa his 3 leu 2 ura 3 trp 1 ade 8 can 1 RAS2a119</td>
<td>M. Wigler (Toda et al., 1985)</td>
</tr>
<tr>
<td>T139-5A-6A</td>
<td>/YEp(CDC25)</td>
<td>M. Wigler (Toda et al., 1985)</td>
</tr>
<tr>
<td>T139-5A-6A</td>
<td>/pC121-8</td>
<td>M. Wigler (Broek et al., 1987)</td>
</tr>
<tr>
<td>T139-5A-6A</td>
<td>/YEp(RAS2a119)</td>
<td>M. Wigler (Broek et al., 1987)</td>
</tr>
<tr>
<td>JT1821</td>
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<td>This work</td>
</tr>
<tr>
<td>JT4100</td>
<td>MATa his 3 leu 2 ura 3 trp 1 ade 8 can 1 pRAS2a119 (LEU2)</td>
<td>This work</td>
</tr>
<tr>
<td>JT5011*</td>
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<td>This work</td>
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<tr>
<td>JT5011</td>
<td>MATa his 3 leu 2 ura 3 trp 1 ade 8 cdc25 : HIS3 pde2 : URA3</td>
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<td>t.s. 321</td>
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<td>M. Jacquet (Camonis et al., 1986)</td>
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<td>M. Jacquet (Camonis et al., 1986)</td>
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<td>This work</td>
</tr>
<tr>
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<td>This work</td>
</tr>
<tr>
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<tr>
<td>JT4300</td>
<td>MATa cdc1-42 ura 3 trp 1 pl25S-P (CDC25 + TRP1)</td>
<td>This work</td>
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* Obtained from a cross between SP1 and a pde2 strain (DJ23-3C, provided by M. Wigler).

Preparation of crude extracts and enzyme assays. Crude enzyme extracts for measurement of invertase and trehalase activity were prepared as described previously (Thevelein & Beullens, 1985). Invertase activity was assayed by the procedure of Goldstein & Lampen (1975) as modified by Celenza & Carlson (1984). Trehalase activity was determined as described before (Thevelein et al., 1983), and trehalase levels were determined by the anthrone method (Ashwell, 1957). Total protein content was determined by the Lowry method.

Reproducibility of results. All experiments were repeated at least twice with consistent results. Representative results are shown.

at 55 °C, cooled on ice and then transferred to a 30°C incubator. Growth was scored after 2 d.

Incubation conditions and cAMP determinations. For cAMP measurements, cells were incubated at a density of 30 mg (wet weight) ml−1 in a reciprocating water-bath at a temperature of 22 °C, 30 °C or 37 °C as indicated in the figure legends. Incubation was carried out in 25 mM-MES/KOH buffer (pH 6) for 10 min or 20 min (in the case of 37 °C) before addition of 100 mM-glucose or 2 mM-2,4-dinitrophenol (DNP). For temperature shift experiments, cells were incubated in YPD, DNP or 25 mM-MES/KOH buffer (pH 6) for 1 h at 22 °C and then rapidly (1–2 min) shifted to 37 °C. cAMP was determined as described previously (Thevelein et al., 1987a). Glucose-induced cAMP responses were routinely followed for 3 min, while DNP-induced responses were followed for 5 min.

Cell transformation and DNA isolation. Transformation of E. coli cells was performed by standard methods (Maniatis et al., 1982). Transformation of yeast cells was done by the lithium acetate method (Ito et al., 1983). Yeast DNA was prepared essentially as described by Struhl et al. (1979). Plasmid DNA was isolated from E. coli (HB101) by the alkaline lysis method (Maniatis et al., 1982).

In vivo 31P-NMR spectroscopy. High-resolution 31P-NMR spectra of living cells were obtained on a Bruker AM-400 WB NMR spectrometer operating in the Fourier transform mode at 161.9 MHz. The spectra were accumulated at 25 °C for 3 min (312 accumulations) after a 10 min pre-incubation period (for temperature equilibration). The phosphocreatine peak was used for calibration (−22.45 p.p.m.). Cells (4 g wet wt) were suspended in 25 mM-MES/KOH buffer (pH 6) (total volume 13 ml) in 20 mm NMR tubes. The cells were continuously bubbled with O2 (or N2 in some control experiments) using two glass capillaries. No substrate was added except in control experiments with mutants having high protein kinase activity (which are known to have low endogenous reserves). They were given 3% glycerol after the normal incubation period and after a second pre-incubation period of 10 min, a new spectrum was accumulated.

Addition of 100 mM-glucose or 2 mM-dinitrophenol (DNP) to wild-type yeast cells grown on non-fermentable carbon sources triggers a rapid increase in the cAMP level (Fig. 1). With glucose, the cAMP increase is always transient so that it takes the form of a cAMP signal. A yeast strain with a disrupted CDC25 gene in which viability was restored by a multicopy plasmid carrying the TPK1 gene has a very low cAMP level (Broek et al., 1987) and did not show a glucose- or DNP-induced cAMP increase (results not shown). To investigate whether the CDC25 protein truly acts as a component of the signalling pathway, or whether it is merely required
for basal cAMP synthesis, we measured the cAMP responses in strains lacking the CDC25 gene but able to synthesize cAMP: cdc25::HIS3 pRAS2val19 and cdc25::HIS3 RAS2val19. The RAS2val19 oncogene product does not depend on the CDC25 protein for its activity, and restores cAMP synthesis in strains lacking CDC25 (Fig. 2). The cAMP level was higher in the cdc25::HIS3 pRAS2val19 strain than in the cdc25::HIS3 RAS2val19 strain, probably owing to the higher copy number of RAS2val19 in the former strain, caused by the presence of the multicopy YEp vector. The RAS2val19 oncogene product itself is unable to transmit the cAMP signal. The strains however still contain RAS1 (and also RAS2 in the cdc25::HIS3 pRAS2val19 strain), which is able to transmit the cAMP signal in the presence of RAS2val19 (Mbonyi et al., 1988). Addition of glucose to cells with a disrupted CDC25 gene did not induce a typical cAMP signal. Neither did DNP cause a cAMP increase comparable to that in wild-type cells (Fig. 2). In the strain containing pRAS2val19 some residual effect on the cAMP level was observed, the reason for which is not known. Control experiments with wild-type cells containing the RAS2val19 oncogene in the genome (Mbonyi et al., 1988) or on a plasmid (Fig. 3) showed that the latter is not responsible, e.g. through increased feedback-inhibition (Nikawa et al., 1987; Mbonyi et al., 1990), for the absence of the cAMP responses.

Disruption of both RAS genes in yeast is lethal, except for instance in strains lacking both the low-affinity (PDE1-encoded) and the high-affinity (PDE2-encoded) phosphodiesterases. In these strains, the basal cAMP level is also restored (Nikawa et al., 1987). In this paper we show that lethality due to CDC25 disruption can be rescued by disruption of the gene encoding high-affinity...
phosphodiesterase. Comparison of the glucose- and DNP-induced cAMP increases in the cdc25 pde2 strain, in which the basal cAMP level is also restored, and the control strain CDC25 pde2 confirmed the importance of CDC25 for induction of the cAMP responses (results not shown). These results show that the CDC25 protein is required for transmission of the glucose-induced cAMP signal. Since the DNP effect also largely disappears when CDC25 is inactivated, intracellular acidification apparently activates the signal transmission pathway at the CDC25 protein or at some point upstream of CDC25. This point, however, must be located downstream of glucose phosphorylation, since yeast mutants lacking sugar kinase activity are only deficient in the glucose-induced cAMP signal, and not in the DNP-induced cAMP increase (BeuMens et al., 1988).

It has been suggested that the CDC25 protein is a specific stimulator of GDP/GTP exchange on the RAS proteins (Broek et al., 1987; Camonis et al., 1986; Daniel, 1986; Daniel et al., 1987; Marshall et al., 1987). Addition of fermentable sugar to derepressed yeast cells probably activates the CDC25 protein, in turn causing a sudden stimulation of GDP/GTP exchange on the RAS proteins. This activates adenyl cyclase, which increases the cAMP level. The rapid decrease after the initial increase might be caused by feedback inhibition by cAMP-dependent protein kinase on one of the components of the signal transmission pathway (Nikawa et al., 1987; Mbonyi et al., 1990).

cAMP levels in the temperature-sensitive mutants cdc25-1 and cdc25-5

Complementation experiments and analysis of the progeny of a cross of the cdc25-1 and cdc25-5 containing strains confirmed that the two genes truly represent alleles (results not shown). The temperature-sensitive mutant cdc25-5 showed a glucose- and DNP-induced cAMP increase at the permissive but not at the restrictive temperature (37 °C) (Fig. 4a). The absence of DNP effect at the restrictive temperature was not due to absence of intracellular acidification as measured by in vivo NMR (results not shown). In contrast, the cdc25-1 mutant showed normal cAMP increases at both the permissive (22 °C) and the restrictive temperature (37 °C) (Fig. 4b). In agreement with the results obtained by Camonis et al. (1986) we found that the cellular cAMP content in cdc25-5 mutants dropped precipitously when the cells were shifted to 37 °C. This was observed both in MES buffer and in rich medium containing either a fermentable or a non-fermentable carbon source (Fig. 5). As opposed to cdc25-5-bearing cells, the cAMP level in the cdc25-1 mutant did not drop, or even increased, when the cells were shifted to the restrictive temperature (37 °C) (Fig. 5). This is in agreement with results obtained previously by Martegani et al. (1986b) and Portillo & Mazon (1986). An increased cAMP level upon shift to high temperature is generally observed in wild-type strains (unpublished results). The experiments with cdc25-1 and cdc25-5 were carried out under exactly the same experimental conditions. Hence, our data confirm the discrepancy between the previous results obtained independently by different groups with the cdc25-1 and cdc25-5 mutants. It cannot be excluded however that the different genetic background in the strains also plays a role, as suggested by recent work from the group of Tatchell (Petitjean et al., 1990) in which the nucleotides modified in the cdc25-1 and cdc25-5 gene were identified, and in which it was claimed that both cdc25-1 and cdc25-5, when present in the same genetic background, resulted...
in a drop of the cAMP level at the restrictive temperature. Unfortunately, the cAMP level in the original cdc25-I strain (t.s. 321) was not investigated. Our results with the original cdc25-I strain demonstrate that in yeast cells cAMP depletion is not required for arrest at the 'start' point of the cell cycle and for subsequent accumulation in the resting state Go.

Is CDC25 the glucose-repressible component of the signal transmission pathway?

Glucose-repressed cells of wild-type strains do not show a glucose-induced cAMP signal, but they show a DNP-induced cAMP increase when they are provided with an adequate ATP level (Beullens et al., 1988; Argüelles et al., 1990). Glucose-repressed cells have a very low ATP level when suspended in buffer (Thevelein et al., 1987b). The absence of both cAMP responses was observed with glucose-repressed cells of the cdc25-5 strain (OL86) incubated at the permissive temperature in buffer (Fig. 6). However, exponential-phase glucose-grown cells of the cdc25-5 mutant, containing a multi-copy plasmid with the CDC25 gene, clearly showed a glucose- and DNP-induced cAMP increase (Fig. 7). This could indicate that the CDC25 protein is the putative glucose-repressible component of the signalling pathway. To check whether the cells with overexpression of CDC25 were still repressed we measured invertase activity and the level of mitochondrial respiration. For the latter, we investigated, by in vitro $^{31}$P-NMR, the ATP level and the intracellular pH in cells suspended in MES buffer and

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**Fig. 5.** cAMP level in the temperature-sensitive strains OL86 (cdc25-5) (●) and t.s.321 (cdc25-I) (○) during incubation at the permissive temperature (22 °C) for 60 min and subsequent shift to the restrictive temperature (37 °C). The experiments were carried out in MES buffer (a), YPD (b) and YPG (c).

**Fig. 6.** cAMP level after addition at the permissive temperature (22 °C) of glucose (●) and DNP (○) to glucose-repressed cells of strain OL86 (cdc25-5).

**Fig. 7.** Glucose-induced cAMP signal at 22 °C (●) and 37 °C (○) and DNP-induced cAMP increase at 22 °C (●) and 37 °C (△) in cells of strain OL97-1-11B pL25 (cdc25-5 pCDC25) grown in YPD (at 37 °C) and harvested in exponential phase.
bubbled with oxygen (den Hollander et al., 1981; Thevelein et al., 1987b). Unexpectedly, overexpression of CDC25 abolished glucose repression as measured by the level of invertase activity (Table 2) and mitochondrial respiration (Fig. 8, Table 3). To solve this problem we made use of the cat1 mutation, which prevents derepression. Because they lack respiration, cat1 mutants are unable to grow on non-fermentable carbon sources. The mutation, however, does not affect derepression of invertase activity, because of the presence of a suppressor allele in the genetic background of the cat1 strains (Entian, 1986; Entian & Zimmerman, 1982; Zimmerman et al., 1977). As is also the case for the cat3 derepression mutant, cat1 cells do not show a glucose-induced cAMP signal, nor do they show a DNP-induced cAMP increase when simply suspended in buffer (Arguelles et al., 1990). Transformation of a cat1 strain with the pCDC25 plasmid did not result in suppression of the deficiency in derepression of mitochondrial functions, and the transformed strain did not grow on non-fermentable carbon sources (results not shown). Investigation of glucose-grown cells of the cat1 pCDC25 strain by in vivo 31P-NMR showed that the cells were repressed, compared to cells of a CAT1 pCDC25 strain (Fig. 8, Table 3).

### Table 2. Invertase activity of cells grown in YPD and harvested in exponential phase or grown in YPD until the glucose in the medium was exhausted ('derepressed cells')

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Invertase activity [nmol min⁻¹ (mg protein)⁻¹]</th>
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<td></td>
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<td>Exponential-phase glucose-grown cells</td>
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<tr>
<td>A364A</td>
<td>Wild-type</td>
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<td>t.s.321</td>
<td>cdc25-1</td>
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### Table 3. ATP level and intracellular pH, as measured by in vivo 31P-NMR, in glucose-grown cells of strains with and without overexpression of CDC25

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<th>Strain</th>
<th>Relevant genotype</th>
<th>ATP level*</th>
<th>Intracellular pH†</th>
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</table>

* In vivo NMR is not well suited for accurate measurement of cellular ATP levels. The difference between the ATP level in repressed cells and derepressed cells however is very clear (Fig. 8). A high ATP level under these experimental conditions is typical for derepressed cells. Because they have respiration they can synthesize ATP from internal carbon sources. Repressed cells can only use fermentation to synthesize ATP and since there is no glucose in the medium, the ATP level of repressed cells under these conditions is low.

† A low intracellular pH under these experimental conditions is typical for repressed cells because they lack ATP. Derepressed cells have a high intracellular pH because they can use their ATP to pump protons out of the cell.

Fig. 8. 31P-NMR spectra of yeast cells grown on YPD and harvested in exponential phase. The cells were incubated in MES buffer at 25 °C and bubbled with oxygen. (a) OL86 (cdc25-5); (b) OL97-1-11B pL25 (cdc25-5 pCDC25); (c) JT4200 (cat1 pCDC25); (d) JT4300 (CAT1 pCDC25). Pi, intracellular phosphate peak; Pi_o, cytoplasmic phosphate peak; Pi_v, vacuolar phosphate peak. ATP_, ATP_\text{p}, ATP_\text{c}; \alpha, \beta, \gamma phosphate groups of ATP.
Table 3). Addition of glucose or DNP to cells of the cat1 strain with overexpression of CDC25 failed to produce cAMP increases (Fig. 9). This indicates that CDC25 is not the putative glucose-repressible component of the signal transmission pathway leading to activation of adenyl cyclase. Control experiments showed that overexpression of CDC25 in other wild-type strains (JT2010 and SP1) also resulted in the abolition of glucose repression, and the presence of glucose- and DNP-induced responses (results not shown).

Two possible explanations can be given for the abolition of glucose repression by overexpression of CDC25. Either overexpression of CDC25 interferes with the activity of a component needed for normal glucose repression (i.e. glucose repression is disrupted) or CDC25 protein acts as a positive stimulator of derepression, with the consequence that overexpression of CDC25 causes derepression under conditions in which the cells are normally repressed. In this respect, it might be recalled that in wild-type cells the level of CDC25 transcript is very low (Camonos et al., 1986; Martegani et al., 1986a). Strain OL86 (cdc25-5) had a relatively low invertase activity under derepressed conditions (Table 2), which is apparently due to the genetic background. As can be seen in Table 2, overexpression of CDC25 resulted in a much higher activity of invertase. This result suggests that CDC25 protein acts as a positive stimulator of derepression and not as an artificial inhibitor of glucose repression.

It is important to note that the glucose-activation pathway of the CDC25/RAS/adenyl cyclase complex is dispensable for basal cAMP synthesis. The latter is shown by the fact that the glucose-activation pathway is glucose-repressible, and that it depends on sugar kinase activity, since mutants without sugar kinase activity display a normal basal cAMP level (Beullens et al., 1988). Fermentable sugar might be considered an unusual trigger for a signal transmission pathway. In mammalian cells the best-understood signal transmission pathways are triggered by hormones and growth factors (Berridge, 1987: Gilman, 1987). In large multicellular organisms, hormones and growth factors are prime regulators of cell metabolism and proliferation. In micro-organisms, on the other hand, nutrients are the prime regulators of metabolism and proliferation. In yeast, in particular, fermentable sugar is the preferred carbon substrate, and it should not surprise us if yeast cells contain a sophisticated system for sensing and responding to the level of fermentable sugar in their environment. In addition to fermentable sugar, however, there may exist other pathways for specific activation of the CDC25/RAS/adenyl cyclase complex. It must also be stressed that glucose-induced activation of cAMP synthesis through the RAS pathway is dispensable for growth on glucose and hence for progression over the ‘start’ point of the yeast cell cycle. The glucose-activation pathway is shut off as soon as the cells are repressed, and therefore it functions only during the transition from the derepressed state to the repressed state. If glucose acts as a stimulator of progression over the ‘start’ point of the yeast cell cycle, it is not through the glucose-activation pathway of the CDC25/RAS/adenyl cyclase complex.

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