Cyclic AMP Can Decrease Expression of Genes Subject to Catabolite Repression in Saccharomyces cerevisiae

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External cyclic AMP (cAMP) hindered the derepression of gluconeogenic enzymes in a pde2 mutant of Saccharomyces cerevisiae, but it did not prevent invertase derepression. cAMP reduced nearly 20-fold the transcription driven by upstream activation sequence (UAS$_{FBP1}$) from FBP1, encoding fructose-1,6-bisphosphatase; it decreased 2-fold the activation of transcription by UAS$_2$FBP1. Nuclear extracts from cells derepressed in the presence of cAMP were impaired in the formation of specific UAS$_{FBP1}$-protein complexes in band shift experiments. cAMP does not appear to act through the repressing protein Mig1. Control of FBP1 transcription through cAMP is redundant with other regulatory mechanisms.

An increase in cyclic AMP (cAMP) acts as a hunger signal in Escherichia coli (14) and is required to relieve repression by glucose (20), while in yeasts cAMP levels are highest in cells using a carbon source such as glucose, which allows efficient glucose (20), while in yeasts cAMP levels are highest in cells derepressed with cAMP, solutions included 5 mM kae2 and responds to the presence of cAMP in the medium. S. cerevisiae derepressed upon glucose exhaustion (2, 3). However, more recent work points to cAMP being involved in catabolite repression in Saccharomyces cerevisiae (22, 23). However, more recent work points to cAMP being involved in catabolite repression and is repressed by glucose (17), as a control gene (Fig. 1). The effect of cAMP on FbPase derepression was transient; after 24 h of derepression in ethanol, FbPase activity was the same in samples with or without cAMP. Northern blotting performed after 4 or 12 h of derepression showed that at 4 h the block of transcription by cAMP was nearly complete, while after 12 h cAMP decreased the FBP1 mRNA level about threefold (Fig. 1). Since we observed that in a pde1 pde2 double mutant the effect of cAMP on FbPase expression was maintained for up to 24 h, we suggest that in the pde1 mutant derepression of the low-affinity phosphodiesterase Pde1 reduces the internal concentration of cAMP and relieves FbPase repression.

The effect of cAMP on transcription was not specific, as shown by using GLK1, which encodes glucokinase and is repressed by glucose (17), as a control gene (Fig. 1).

The FBP1 promoter comprises two UAS elements (24, 26, 30) and an upstream repressing sequence able to bind the regulatory protein Mig1 (21, 25). cAMP could act by blocking transcription through cAMP is redundant with other regulatory mechanisms.

### Table 1. Effect of external cAMP on the derepression of different enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (nmol/min/mg of protein)*</th>
<th>Ethanol</th>
<th>Ethanol + 5 mM cAMP</th>
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<tbody>
<tr>
<td>FbPase</td>
<td>61 ± 6</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxykinase</td>
<td>270 ± 86</td>
<td>43 ± 15</td>
<td></td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>120 ± 22</td>
<td>32 ± 13</td>
<td></td>
</tr>
<tr>
<td>NAD-dependent glutamate dehydrogenase</td>
<td>210 ± 23</td>
<td>90 ± 27</td>
<td></td>
</tr>
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</table>

* Enzymatic activities were measured in extracts of S. cerevisiae OL556 (pde2) after 12 h of derepression on ethanol in the presence or absence of cAMP, as described in the text. Values are averages and standard deviations of at least six independent experiments.

† Activity in repressed cells is indicated in parentheses.
activation through the UASs or by interfering with the release of Mig1 inhibition which occurs upon glucose removal (8). To investigate possible targets for cAMP, we used different fusions with the reporter gene lacZ: either the complete FBP1 promoter or the UAS1 (−450 to −407) or UAS2 (−2507 to −2489) element was fused with lacZ. As shown in Table 2, cAMP decreased the expression of FBP1-lacZ and also had a strong effect (over 15-fold repression) on UAS1-lacZ; for UAS2-lacZ, the decrease in expression was only two to threefold. To test whether cAMP could act by converting Mig1 into a constitutive repressor, we looked at the effect of cAMP on a pde2 strain where the MIG1 gene had been interrupted and found that cAMP was as effective in blocking FBP1 expression in it as in the corresponding MIG1 strain.

To examine the effect of cAMP on the transcription factors binding the UASs, we tested by a band shift assay nuclear extracts from cells derepressed in the presence of cAMP; the specific DNA-protein complex formed with UAS2 was not observed under these conditions, and only one of the specific complexes was formed with UAS1, a pattern similar to that found with extracts from repressed cells (Fig. 2). We checked that cAMP added during the band shift assay had no effect on the formation of DNA-protein complexes; within the cell, however, cAMP could interfere with the synthesis of FBP1-activating proteins or trigger their modification, decreasing their capacity to bind in vitro to the corresponding UASs. The relevant target for the protein kinases activated by cAMP has not been identified; a candidate would be the transcription factor Cat8, required for the derepression of gluconeogenic enzymes (16, 28) and with two potential sites for phosphorylation by the cAMP-dependent protein kinases. Although the transcription factors Msn2 and Msn4 control many genes induced at the diauxic transition, they are not required for the derepression of isocitrate lyase (2), and therefore, they are probably also not involved in FBP1 transcription.

To investigate whether cAMP may be the main trigger for catabolite repression of certain genes, we have utilized a yeast strain, derived from RS13-58A-1h (5), with a low protein kinase activity independent of cAMP levels. In S. cerevisiae JF908 (MATa ade8 his3 leu2 trp1 ural3 tpklw tpk2::HIS3 tpk3::TRPI bcy1::LEU2 GSY2-lacZ::URA3), provided by J. M. FIG. 1. FbPase mRNA is responsive to the presence of cAMP in the derepressing medium. S. cerevisiae OL556 (pde2) was grown in YPD and derepressed in YP-ethanol (YPEt) for 4 or 12 h in the presence or absence of 5 mM cAMP. Northern analysis was performed by loading 15 μg of total RNA in each lane and using labelled FBP1 and GLK1 probes. The bottom row shows methylene blue staining of 18S rRNA.

FIG. 2. Effect of the presence of cAMP in the derepressing medium on the capacity of nuclear extracts to form specific DNA-protein complexes with UAS1 (A) and UAS2 (B). Nuclear extracts from S. cerevisiae OL556 (pde2) were prepared from repressed cells (YPD) or cells derepressed in YP-ethanol (YPEt) for 12 h in the presence or absence of 5 mM cAMP. Twenty (UAS1) or 40 μg (UAS2) of nuclear proteins was used in each case. When indicated, a 100-fold excess of the unlabelled oligonucleotides was added as competitor DNA. Specific complexes are indicated with arrows.

<table>
<thead>
<tr>
<th>Fusion gene</th>
<th>Activity of β-galactosidase (nmol/min/mg of protein)</th>
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<tbody>
<tr>
<td>FBP1-lacZ</td>
<td>413 ± 23</td>
</tr>
<tr>
<td></td>
<td>46 ± 6 (4)</td>
</tr>
<tr>
<td>UAS1-lacZ</td>
<td>1,120 ± 85</td>
</tr>
<tr>
<td></td>
<td>60 ± 18 (10)</td>
</tr>
<tr>
<td>UAS2-lacZ</td>
<td>580 ± 80</td>
</tr>
<tr>
<td></td>
<td>260 ± 80 (14)</td>
</tr>
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</table>

*S. cerevisiae OL556 (pde2) was transformed (19) with plasmids containing the indicated fusion genes. For FBP1-lacZ, we used the pJJ11b plasmid (30), and for UAS1-lacZ and UAS2-lacZ, we used the plasmids described by de Mesquita et al. (7).

β-Galactosidase was measured (30) after 12 h of derepression on ethanol in the presence or absence of cAMP, as described in the text. In all cases FbPase was measured in parallel and was found to be repressed at least 20-fold by cAMP. Values are averages and standard deviations of at least three independent experiments.

Activity in repressed cells is indicated in parentheses.
François, both FbPase and NAD-dependent glutamate dehydrogenase were repressed by glucose as in a wild-type strain and derepressed upon incubation in an ethanol medium. It is therefore clear that derepression of FbPase (and of other enzymes) does not depend only on changes in cAMP levels. This is consistent with the results of Yin et al. (31), which suggested that different signalling pathways were involved in the response to glucose of FBP1 and PCK1 transcription. Control by cAMP would then be at least partially redundant with other regulatory mechanisms.

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