High cAMP levels antagonize the reprogramming of gene expression that occurs at the diauxic shift in *Saccharomyces cerevisiae*

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In order to analyse the involvement of the cAMP pathway in the regulation of gene expression in *Saccharomyces cerevisiae*, we have examined the effect of cAMP on protein synthesis by using two-dimensional gel electrophoresis. cAMP had only a minor effect on the protein pattern of cells growing exponentially on glucose. However, it interfered with the changes in gene expression normally occurring upon glucose exhaustion in yeast cultures, maintaining a protein pattern typical of cells growing on glucose. This effect was accompanied by a delay before growth recovery on ethanol. We propose a model in which the cAMP-signalling pathway has a role in the maintenance of gene expression, rather than in the determination of a specific programme. A decrease of cAMP would then be required for metabolic transitions such as the diauxic phase.

Keywords: gene regulation, cAMP-dependent protein kinase (PKA) effects, cAMP pathway

INTRODUCTION

In the yeast *Saccharomyces cerevisiae*, the cAMP-signalling pathway is involved in a wide variety of cellular processes and interferes with the cell's response to environmental variations, as deduced from the study of mutants altered in this pathway. cAMP is required for normal cell growth; its absence blocks cell division in early G₁ phase. In the absence of the regulatory subunit (Bcy1p) or in the excess of cAMP, a high level of constitutive cAMP-dependent protein kinase (PKA) activity allows growth and division but is lethal to cells subjected to heat-shock or nitrogen starvation. It prevents the accumulation of trehalose and glycogen. In addition, sporulation is blocked but pseudo-hyphal growth is facilitated (Boy-Marcotte *et al.*, 1987; Gimeno *et al.*, 1992; Kataoka *et al.*, 1984; Matsumoto *et al.*, 1985; Tatchell, 1993; Toda *et al.*, 1985).

Although the components of this pathway are well known (for a review, see Tatchell, 1993), its activation and the nature of the regulatory signals are not clearly understood. The only known signals which activate this pathway are the rapidly fermentable sugars such as glucose, fructose

and mannose (Van Aelst *et al.*, 1990). Two- to threefold variations of the cAMP intracellular concentration have been reported, depending on the carbon source (François *et al.*, 1987; Russel *et al.*, 1993) and on the cell division cycle (Watson & Berry, 1977). It is not yet understood how this pathway controls such a large number of cellular processes, since very few direct targets of the PKA have been identified to date (Van Der Plaats, 1974; François *et al.*, 1984; Thevelein, 1984; Kinney & Carman, 1988; Cherry *et al.*, 1989).

It has been shown in mammalian cells that when PKA is activated by cAMP, the catalytic subunit moves to the nucleus (Adams *et al.*, 1991). In mammalian cells, cAMP-dependent transcriptional regulation occurs through cAMP-response elements (CREs), which are binding sites for CRE-binding factors (CREBs). Phosphorylation by PKA and dephosphorylation seem to be important mechanisms in the regulation of CREB activity (Brindle & Montminy, 1992). In *S. cerevisiae*, negative effects of cAMP have been reported for numerous genes, including *ADH2*, *CTT1*, *GAC1*, *GPH1*, *GSY2*, *IME2* and *UBI4* (Denis *et al.*, 1992; François *et al.*, 1992; Hardy *et al.*, 1994; Marchler *et al.*, 1993; Tanaka *et al.*, 1988) and genes induced by heat-shock, such as *HSC82*, *HSP26*, *HSP104*, *SSA1* and *SSA3* (Boorstein & Craig, 1990; Engelberg *et al.*

Abbreviations: PKA, cAMP-dependent protein kinase; 2-D, two-dimensional.

al., 1994b). It has been shown that the negative effect of cAMP on the heat-shock response does not involve the heat-shock factor. For *CTT1* and *SSA3*, a similar DNA-responsive element, sensitive to cAMP, has been identified upstream of the *UBI4* and *GAC1* genes. Nevertheless, no transcriptional regulators have yet been identified which could be responsible for this negative effect of cAMP. Positive regulation by cAMP has also been reported recently for transcription of ribosomal proteins. In this case, the positive effect is mediated by the transcriptional activator RAP1 (Klein & Struhl, 1994). A positive effect of cAMP on transcription mediated by GCN4 has also been reported (Engelberg *et al.*, 1994a).

In order to better define the role of cAMP on gene expression in glucose-grown cells, we have compared the protein synthesized in cells grown with and without added cAMP by incorporation of [³⁵S]methionine and protein separation by two-dimensional (2-D) gel electrophoresis. This approach has been fruitfully applied to determine several sets of co-regulated genes in *S. cerevisiae*, such as heat-shock genes and glucose-repressed genes (Bataillé *et al.*, 1988, 1991). We followed the change in protein synthesis until glucose was exhausted from the medium. Whereas little change was observed between cells growing exponentially on glucose with and without cAMP, the numerous changes in patterns of proteins synthesized after glucose exhaustion were mostly prevented by the presence of cAMP in the culture medium.

METHODS

Yeast strain. The OL556 diploid strain used in these experiments was obtained by conjugating OL554 (α *cdc25-5 his3 leu2 rca1/pde2 ura3*) and OL555 (α *cdc25-5 his3 leu2 trp1 rca1/pde2 ura3*). These two haploids are meiotic products of the cross of S150-2B (α *his3 leu2-112 trp1-289 ura3-52*; Daignan-Fornier *et al.*, 1988) with OL520-2 (α *cdc25-5 his3 leu2 rca1/pde2 ura3*; our laboratory). In addition to their genotypes, these haploid strains have also been selected for their permeability to the chromogenic substrate X-Gal by the blue colour of the clones when transformed with *lacZ* as a reporter gene inserted into a plasmid.

Culture conditions. Supplemented minimal medium, YNBS, contained 0.17% yeast nitrogen base without ammonium sulfate and amino acids, 0.5% ammonium sulfate, 2% (w/v) glucose, 25 µg inositol ml⁻¹, 800 µg leucine ml⁻¹, 100 µg isoleucine ml⁻¹, 100 µg valine ml⁻¹, 300 µg histidine ml⁻¹, 100 µg uracil ml⁻¹ and 85 mM succinate/NaOH, pH 5.8. Complete medium contained 1% (w/v) yeast extract, 2% (w/v) bactopectone and 1% (w/v) glucose (YPD) or 2% (v/v) ethanol (YPE). An overnight preculture grown at 26 °C in the required medium in the absence of cAMP was used to inoculate the culture at 10⁶ cells ml⁻¹. Growth was continued (at the indicated temperature) in the absence or the presence of 3 mM cAMP.

Measurement of cell density and budding and estimation of viability. The cell density in the culture was estimated either by measuring the OD₇₁₀ with a Jenway 6061 colorimeter, or by counting the cell number under the microscope with a haemocytometer. In this latter case, a minimum of 200 budded and unbudded cells were counted after separation by mild sonication. Viability was determined by spreading appropriate dilutions of the sonicated culture on complete solid medium and counting c.f.u. after 3 d at 26 °C.

Soluble protein content of cells. A sample of culture containing 2–5 × 10⁸ cells was withdrawn, washed once and concentrated in 1 ml ice-cold 100 mM Tris/HCl, pH 8, containing 1 mM dithiothreitol and 20% (v/v) glycerol (cell-breakage buffer). Cell concentration of the concentrated sample was measured by counting under the microscope. For each sample, three independent extractions were performed on 300 µl of the concentrated sample in cell-breakage buffer by vortexing at 4 °C with 300 mg glass beads (0.45–0.5 mm diameter) until less than 1% of intact cells were still visible by light microscopy. Extracts were clarified by centrifuging for 15 min in a microfuge at 18000 g at 4 °C. Protein concentration was measured in each extract by using the Bradford (1976) dye-binding assay (Bio-Rad reagent).

Glucose measurement. The concentration of glucose in the culture medium was measured enzymically with glucose oxidase, using the Sigma Diagnostic Glucose reagent kit no. 510-A.

Radioactive labelling of proteins, preparation of cell extracts and 2-D gel electrophoresis were as described by Boucherie *et al.* (1995).

RESULTS

Effect of cAMP on growth of strain OL556 in minimal glucose medium

The intracellular level of cAMP can be modulated in diploid strain OL556 homozygous for *cdc25-5* and *rca1/pde2* mutations. In this strain, cAMP is not synthesized at 37 °C due to the *cdc25-5* mutation (Camonis *et al.*, 1986). Resulting growth arrest can be rescued by exogenously added cAMP as a consequence of the *rca1/pde2* mutation which affects the high-affinity phosphodiesterase (Wilson *et al.*, 1993). In the absence of this mutation, very high concentrations of exogenous cAMP are insufficient to complement the defect in cAMP synthesis. However, when the high-affinity phosphodiesterase was inactive, the intracellular level of cAMP was 50 µM when 3 mM cAMP was added exogenously, this being sufficient to complement the defect of cAMP synthesis (Wilson *et al.*, 1993). In our strain (OL556), a sufficient level of cAMP was produced at the permissive temperature to allow cell division. When 3 mM cAMP was added to the medium with 2% glucose at 28 °C, a slight increase of the generation time was observed (Fig. 1a; Table 1). This prolonged cell cycle resulted in the formation of larger cells, leading to a higher coefficient of turbidity (Table 1) as already reported (Baroni *et al.*, 1989). We found that the protein content of these cells was three- to fourfold higher with cAMP than without (Table 1) and, as a consequence, the number of cells generated for a given amount of glucose was fourfold lower in cAMP-treated cultures (Fig. 1a). These phenotypes are a mark of the high level of PKA activity due to the *rca1/pde2* mutation and the addition of cAMP. Growth arrest occurred simultaneously with the exhaustion of glucose from the medium in both the presence and absence of cAMP (Fig. 1b) and corresponded with diauxic lag phase (Lewis *et al.*, 1993). When glucose was exhausted, the ratio of budded cells, which decreased to less than 10% in untreated cells, was maintained up to 35% in the cAMP-treated culture. High cell viability was maintained

Fig. 1. (a) Growth of the OL556 strain in YNBS medium in the absence (open symbols) or in the presence (filled symbols) of 3 mM cAMP. Culture conditions are described in Methods. $\square$, $\blacksquare$, Cells ml^{-1} ; $\triangle$, $\blacktriangle$, percentage of budded cells. Arrows indicate times, in exponential growth phase and lag phase, at which samples have been withdrawn to perform the measurements indicated in Table 1 and radioactive labelling of proteins. (b) Time-course of glucose depletion in the culture of strain OL556 in YNBS in the absence (open symbols) or the presence (filled symbols) of 3 mM cAMP. $\square$, $\blacksquare$, Cells ml^{-1} ; $\circ$, $\bullet$, glucose concentration in the medium.

throughout the experimental period irrespective of cAMP addition (Table 1).

Pattern of protein synthesis during exponential growth and at the diauxic transition

In order to assess the effect of cAMP on gene expression during growth on glucose, we examined the 2-D protein patterns after a pulse of [^{35}S]methionine. Two different growth stages were considered: exponential growth phase and the arrested phase after glucose exhaustion. For each stage, cells grown in the absence or the presence of cAMP were compared. We used an improved 2-D gel electrophoresis method derived from the O'Farrell technique, allowing resolution of soluble proteins with a pI ranging from 4.2 to 6.8 and with an M_r between 15 000 and 150 000 (Boucherie *et al.*, 1995).

As shown in Fig. 2, the patterns from cells growing exponentially on glucose, without (Fig. 2a) or with (Fig. 2b) cAMP, were very similar. These patterns closely resembled the pattern of wild-type cells growing exponentially on a fermentable carbon source. Under these conditions, the major spots corresponded to glycolytic enzymes (Boucherie *et al.*, 1995). Some of them are numbered on the patterns for reference and are listed in Table 2. Differences concerning only three polypeptides (spots 11, 12 and 13) emerged from the comparison of Fig. 2(a) and (b). Spots 11 and 12 were absent in cells grown without cAMP. The polypeptide corresponding to spot 13 was synthesized under both culture conditions but its relative rate of synthesis was markedly increased in the presence of cAMP. None of these spots have yet been identified.

In contrast, the pattern of protein synthesis after glucose exhaustion was dramatically different between cells grown without (Fig. 2c) or with (Fig. 2d) cAMP. Two experiments, performed 120 min and 180 min after growth arrest (Fig. 1a), gave a similar pattern. They differed only in the amount of protein synthesized, which was reduced

Table 1. Effect of cAMP on OL556 cells grown in YNBS medium

Percentage of budding cells and cell viability, protein content in the culture and cell protein content have been measured on samples E, E+cAMP, S_2 and S_2 +cAMP withdrawn from the culture described in Fig. 1, as outlined in Methods.

Growth phase	Generation time (min)*	Budding (%)	Viability (%)	$10^{-7} \times$ Cells per OD_{710} unit†	Soluble protein [mg (ml culture) $^{-1}$]‡	Cell soluble protein content (pg cell $^{-1}$)‡
E	126	71	92	1.4 ± 0.16	0.087 ± 0.021	5.5
E+cAMP	165	85	100	0.82 ± 0.08	0.114 ± 0.003	15.1
S_2		9	62	1.45	0.47 ± 0.03	4.85
S_2 +cAMP		35	70	0.71	0.59 ± 0.04	18.3

* Generation times were measured on the growth curves in Fig. 1.

† The ratio ($\pm$ standard deviation) of the cell concentration to the turbidity in exponential growth phase is the mean of six values measured during this exponential growth phase. In stationary phase, the ratio indicated is the one measured in samples S_2 and S_2 +cAMP.

‡ These values are the means of three independent measurements.

Fig. 2. 2-D protein patterns from cells labelled at times indicated by arrows (E, S_1 , S_2) on Fig. 1. Two millilitres of samples E and E+cAMP were labelled for 30 min, whilst 0.3 ml samples S_1 and S_2 and 0.6 ml samples S_1 +cAMP and S_2 +cAMP were labelled for 50 min with 3.7×10^9 Bq [35 S]methionine ($> 3.7 \times 10^{13}$ Bq mmol $^{-1}$, Amersham). After the labelling period, the cells were washed and extracted in 200 μ l extraction buffer. In this experiment, methionine incorporated was: in E, 4.75×10^7 c.p.m. in 2.9×10^7 cells; in E+cAMP, 4.8×10^7 c.p.m. in 1.65×10^7 cells; in S_1 , 3.6×10^7 c.p.m. in 3×10^7 cells; in S_1 +cAMP, 7.2×10^6 c.p.m. in 1.9×10^7 cells. Separation of proteins was done by 2-D gel electrophoresis. An aliquot (15 μ l) of each extract was loaded on the first dimension gel. Radioactivity on the gels was revealed using a PhosphorImager (Molecular Dynamics). Adjustment of image intensities has been done to have approximately the same intensity for the actin spot on the four images. (a) Protein pattern from aliquot E; (b) protein pattern from aliquot E+cAMP; (c) protein pattern from aliquot S_1 ; (d) protein pattern from aliquot S_1 +cAMP. Spots indicated by black arrowheads on (a) and (d) correspond to proteins listed in class A of Table 2. White arrowheads on (b) and (d) indicate spots corresponding to proteins induced by cAMP. Circles on (c) indicate reduction or disappearance of spots 2, 7, 10, 13 and 15. Black arrowheads and white arrowheads on (c) indicate, respectively, major glucose-repressed proteins and stress proteins present on (c) and absent on (a).

after 180 min. The pattern of protein synthesis was markedly different from exponentially growing cells (Fig. 2a, c) 120 min after growth arrest without cAMP as previously reported by Bataillé *et al.* (1991) and Boucherie (1985). In particular, glycolytic enzymes were either absent, e.g. enolase B (spot 7) and glyceraldehyde-3-phosphate dehydrogenase B (spot 15), or only detectable as minor spots, e.g. pyruvate decarboxylase (spot 2), fructose-1,6-bisphosphate aldolase (spot 10) and glyceraldehyde-3-phosphate dehydrogenase C (spot 14). Synthesis of new polypeptides was induced at this time. These belonged to two sets of proteins, proteins subject to glucose repression and stress-induced proteins (Bataillé *et al.*, 1991).

As shown in Fig. 2(d), the presence of cAMP in the culture medium interfered with most of the changes induced by glucose exhaustion. The relative rates of synthesis of individual proteins could be estimated roughly by comparison of the intensity of the corresponding spots to the intensity of the actin spot. From this comparison, it was observed that the relative rate of synthesis was maintained for most of the major proteins, normally repressed after glucose exhaustion. A subset of these proteins, chosen because they have been already identified, is indicated in Fig. 2(a, d) and is reported in Table 2 (class A). The proteins correspond mostly to glycolytic enzymes. Among the proteins synthesized during exponential phase, two proteins, enolase A (spot 6)

Table 2. Proteins whose synthesis is modified by cAMP

Spot number or letter mentioned in this table corresponds to the number or letter in Fig. 2. Identification of the spots has been previously reported by Bataillé *et al.* (1991), Boucherie (1985) and Boucherie *et al.* (1995). Their presence or their absence on 2-D maps (Fig. 2a-d) is indicated by + or -. The number of crosses represents the spot intensity compared to the same spot in Fig. 2(a). CCR, proteins whose synthesis is repressed by glucose; HS, proteins whose synthesis is induced by heat-shock. *, Unidentified protein.

Number on the maps	Gene	Protein	E	E+ cAMP	S	S+ cAMP	Regulation
Class A							
1	<i>MET6</i>	Methionine synthase	+	+	-	+	
2	<i>PDC1</i>	Pyruvate decarboxylase	+	+	-/+	+	
3	<i>PGI1</i>	Phosphoglucosomerase	+	+	-	+/-	
4	<i>ZWF1</i>	Glucose-6-phosphate dehydrogenase	+	+	-	+/-	
5	<i>HXK2</i>	Hexokinase PII	+	+	-	+	
6	<i>ENO1</i>	Enolase A	+	+	-	+++	
7	<i>ENO2</i>	Enolase B	+	+	-	+	
8	<i>SAM1</i>	S-Adenosyl-methionine synthetase	+	+	-	+	
9	<i>TIF1</i>	Translation initiation factor 4A	+	+	-	+/-	
10	<i>FBA1</i>	Fructose-bisphosphate aldolase	+	+	-	+	
14	<i>TDH3</i>	Glyceraldehyde-3-phosphate dehydrogenase C	+	+	+/-	+	
15	<i>TDH2</i>	Glyceraldehyde-3-phosphate dehydrogenase B	+	+	-	+	
Class B							
10	*	*	-	-/+	-	+	
2	*	*	-	+	-	+	
13	*	*	-	-/+	-	+++	
Class C							
a	*	*	-	-	+	-	CCR
b	<i>ICL1</i>	Isocitrate lyase	-	-	+	-	CCR
c	*	*	-	-	+	-	CCR
d	*	*	-	-	+	-	CCR
e	*	*	-	-	+	-	CCR
f	<i>HXK1</i>	Hexokinase PI	-	-	+	-	CCR/HS
g	*	*	-	-	+	-	HS
h	*	*	-	-	+	-	HS
i	*	*	-	-	+	-	HS
j	*	*	-	-	+	-	CCR
k	*	*	-	-	+	-	CCR
l	*	*	-	-	+	-	CCR
m	<i>ADH2</i>	Alcohol dehydrogenase II	-	-	+	-	CCR
n	*	*	-	-	+	-	CCR
o	*	*	-	-	+	-	HS

and alcohol dehydrogenase I, seemed at a first glance to have a different behaviour. The relative rate of synthesis of enolase A was higher after glucose exhaustion than during exponential phase. Alcohol dehydrogenase I, which corresponded to a major spot during exponential phase, became undetectable after glucose exhaustion, even in the presence of cAMP. Previous investigations had shown that the relative rate of synthesis of enolase A progressively increased during the time-course of growth on glucose. During the same period, the relative rate of

synthesis of alcohol dehydrogenase I progressively decreased (Bataillé *et al.*, 1991; Boucherie, 1985). These observations concerning enolase A and alcohol dehydrogenase I probably reflect changes in the relative level of synthesis of these enzymes normally occurring prior to glucose exhaustion rather than the existence of a particular behaviour of these enzymes in response to cAMP.

A second dramatic effect of cAMP was to prevent the synthesis of proteins normally induced in response to

Fig. 3. YPD medium in the absence (□) or in the presence (■) of cAMP was inoculated with 10^6 cells ml⁻¹ using a preculture of strain OL556 in YPD at 27 °C. Arrows indicate the time at which aliquots were diluted 10 times in prewarmed YPE $\pm$ 3 mM cAMP. (a) Growth in YPD; (b) growth in YPE at 27 °C after dilution from the YPD culture.

glucose exhaustion. Comparison of Fig. 2(c) and (d) gave evidence that in the presence of cAMP, none of the proteins subject to glucose repression were induced 2 h after glucose exhaustion. In the same way, induction of synthesis of stress-induced proteins was not detected. The same pattern was observed 3 h after glucose exhaustion in the presence of cAMP (data not shown). Some of these non-inducible proteins are listed in Table 2, class C: 11 are the products of glucose-repressed genes, among them isocitrate lyase, hexokinase PI and alcohol dehydrogenase II; 4 are the products of stress-induced genes.

Finally, comparison of protein patterns of cells which have exhausted their glucose supply in the absence or the presence of cAMP also showed differences which were not related to changes normally occurring when glucose is consumed. Spots 11, 12 and 13, already detectable in cells growing exponentially on glucose, were abundantly synthesized in the presence of cAMP and were undetectable in the absence of cAMP (Fig. 2d; Table 2, class B).

cAMP delays, but does not prevent, post-diauxic growth

Growth of the OL556 strain on rich medium containing 1% glucose in the presence or absence of 3 mM cAMP was followed to test the ability of the cells to switch to

respiratory growth on ethanol. YPE medium was used in preference to minimal medium because the generation time on ethanol of this strain was shorter on rich medium than on minimal medium. Growth was performed at 27 °C because this strain is thermosensitive at 30 °C in ethanol medium, due to the *cdc25-5* mutation. In the absence of cAMP, a delay (2–3 h) was observed before a doubling of the cell number. In the presence of 3 mM cAMP, this delay was increased to 10 h (Fig. 3). When the arrested cells were diluted in YPE without cAMP, no lag was observed before recovery of the exponential growth. When cAMP was present, recovery of exponential growth was observed after a lag of 5 h with a generation time even shorter than in the absence of cAMP (Fig. 3). These observations indicate that cAMP delayed the switch to respiratory growth, but that growth on ethanol was not prevented by cAMP. Moreover, cAMP complemented the weak growth defect of the OL556 strain due to the *cdc25-5* mutation.

DISCUSSION

The use of the *rca1/pde2* mutant and exogenous cAMP permits comparison of the properties of the same strain having a wild-type intracellular level of cAMP (in the absence of exogenous cAMP) and having a high cAMP level (in the presence of exogenous cAMP). The addition of cAMP to the medium leads to large cells and an increase in cell protein content, which is a mark of a high intracellular cAMP level. These cells remain viable and divide normally, which suggests that this high cAMP level has no deleterious effects. Moreover, the phenotypes of nitrogen starvation, heat-shock and sensitivity, as well as reduction in glycogen accumulation, usually associated with deregulation of the PKA, are weaker in this strain grown in the presence of cAMP than in a *bcy1* strain (data not shown). This strain has already been used to demonstrate cAMP effects on various cellular phenomena (Baroni *et al.*, 1989; Bissinger *et al.*, 1989; Tokiwa *et al.*, 1994).

In this report, we have analysed the changes in the protein synthesis patterns in *S. cerevisiae* induced by exogenously added cAMP during and after growth on glucose as sole carbon source. Use of an improved 2-D gel electrophoresis system allowed the simultaneous visualization of a large subset of cellular proteins, in the pI range 4.2 to 6.8 and *M_r* range from 15 000 to 150 000, which is representative of the synthetic capability of the cell as it corresponds to 30% of total soluble proteins (Boucherie *et al.*, 1995). Almost 100 spots currently are identified, and the number of identified spots is rapidly increasing with analyses associated with the yeast genome sequencing projects.

During exponential growth on glucose, the usual pattern of gene expression was observed with a high level of expression of genes involved in glycolysis. In the *pde2/rca1* mutant, the presence of cAMP in the medium did not cause large modifications in the protein pattern of the cell, which again indicates that the cell physiology was

not greatly modified by the increased intracellular cAMP level. We detected only three differences corresponding to proteins induced in cells grown with cAMP in the medium. Synthesis of these proteins was maintained after glucose exhaustion. Their identification would be of interest because they might be critical effectors of the cAMP pathway. A two- to threefold stimulation of the transactivation potential of RAP1 by PKA has been reported (Klein & Struhl, 1994). Nevertheless, we have not detected any consequences of this control. Ribosomal proteins, whose transcription is activated by RAP1, are not included in our analysis because they are too basic. A possible explanation, for other non-ribosomal proteins under RAP1 control, is that the presence of glucose leads to an intermediate level phosphorylation of RAP1 and other transcriptional activators, and that the small variation due to the addition of cAMP was not detected in our analysis.

The most dramatic observation of our analysis was the difference in the pattern of protein synthesis after glucose exhaustion with and without cAMP. Consumption of glucose is known to trigger several modifications of gene expression, and the pattern observed without cAMP was similar to that previously described in other strains (Bataillé *et al.*, 1991). There was a general reduction in protein synthesis with a marked repression of glucose-induced proteins, while glucose-repressed proteins and heat-shock proteins were induced, at least transiently. The presence of cAMP in glucose-deprived medium interfered dramatically with these changes. cAMP did not prevent the drop in total synthetic capability, as judged from the incorporation of radioactive methionine, but it maintained the protein distribution of glucose-grown cells as if glucose was still present. Indeed glycolytic enzymes were not repressed and all the known glucose-repressed proteins were not induced. These results illustrate the interference of the Ras/cAMP pathway with the expression of genes controlled by glucose. Transient induction of some heat-shock proteins, which is normally associated with glucose exhaustion, was also prevented. This observation is in agreement with previous data indicating a negative effect of cAMP on the transcriptional heat-shock response of several genes. We suspect that the genes which encode these proteins belong to the class of genes induced by a variety of stresses and negatively regulated by cAMP through stress response elements (Marchler *et al.*, 1993).

This is the first time that such a large pleiotropic effect on gene expression has been reported for cAMP-treated cells. The major qualitative changes were observed at the end of growth on glucose. This stage corresponds to the diauxic shift, during which cells switch from the fermentative to the respiratory programme of gene expression. At the end of growth on glucose, mRNA of positive regulators of the cAMP pathway such as *CDC25*, *RAS2* and *CDC35* is reduced, whereas mRNA of negative regulators such as *IRA1* and *IRA2* accumulates, leading to a reduction in cAMP levels within cells (François *et al.*, 1984; Russel *et al.*, 1993). This reduction is required for the diauxic shift, since this transition was delayed by an excess of cAMP in

strain OL556, as described in this paper, and even prevented in other strains (Russel *et al.*, 1993). Our results further establish the involvement of cAMP regulation in this transition and can explain the growth defect on acetate of *bcy1* strains (Toda *et al.*, 1987). When a high level of cAMP was artificially maintained, changes in gene expression were delayed or prevented as documented in this report. These results lead us to conclude that this change in gene expression pattern, which requires a drop in PKA activity, is essential for the switch from fermentative to non-fermentative conditions. Nevertheless, the cAMP pathway does not impair growth on respiratory carbon sources as we describe in this paper and it is even essential for growth on any carbon source. Thus, a decrease of the cAMP level is important for the occurrence of the diauxic transition but not for steady-state growth on non-fermentable carbon sources. Taking these data into account, we propose the following model for the role of the cAMP-signalling pathway on regulation of gene expression. It will reinforce the activity or the stability of many transcriptional factors by modification, rather than determining their activity. In this way, it will stabilize a programme of gene expression defined by a set of transcriptional regulators adapted to a defined environment. If the environment changes, by glucose exhaustion for example, these cAMP-dependent modifications have to be removed by a transient reduction of the cAMP pathway activity to allow these transcriptional regulators to change their activity in response to the new environmental conditions. If cAMP is maintained at a high constitutive level, it will antagonize the change in gene expression. As an illustration, we observed that the maintenance of cAMP at a high level maintains the programme of gene expression adapted to glucose conditions and prevents the setting up of the programme adapted to non-fermentable carbon sources. Such a role for cAMP implies a very pleiotropic effect, acting either positively or negatively on many transactivators and repressors. It explains the pleiotropic phenotype associated with deregulation of this pathway and reinforces the view that this pathway serves as a signalling pathway during a transition period to adapt cells to new conditions (Thevelein, 1994). In this model, the phenotype of sensitivity to ammonium starvation and heat-shock, observed in mutants where the PKA is hyperactive, can be re-interpreted as the inability of the yeast cell to change its gene expression programme in response to variations in the environment. This model is not contradictory with the observation that in *tpk^wbcy1* mutants, the cellular response to environmental changes is apparently normal although the pathway is no longer regulated by cAMP. In this case, the phosphorylation is maintained at a very low level and the change in the gene expression programme is less dependent on a decrease in PKA activity.

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