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Cyclic AMP controls the plasma membrane H^+-ATPase activity from *Saccharomyces cerevisiae*

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The thermosensitive G_1-arrested *cdc*35-10 mutant from *Saccharomyces cerevisiae*, defective in adenylate cyclase activity, was shifted to restrictive temperature. After 1 h incubation at this temperature, the plasma membrane H^+-ATPase activity of *cdc*35-10 was reduced to 50%, whereas that in mitochondria doubled. Similar data were obtained with *cdc*25, another thermosensitive G_1-arrested mutant modified in the cAMP pathway. In contrast, the ATPase activities of the G_1-arrested mutant *cdc*19, defective in pyruvate kinase, were not affected after 2 h incubation at restrictive temperature. In the double mutants *cdc*35-10 *cas*1 and *cdc*25 *cas*1, addition of extracellular cAMP prevented the modifications of ATPase activities observed in the single mutants *cdc*35-10 and *cdc*25. These data indicate that cAMP acts as a positive effector on the H^+-ATPase activity of plasma membranes and as a negative effector on that of mitochondria.

ATPase, H^+-; cyclic AMP; Plasma membrane; Cell cycle; ATPase, mitochondrial; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

In yeast cells, cyclic adenosine 3',5'-monophosphate (cAMP) controls a variety of metabolic and physiological functions [1]. As in mammalian cells, this control is exerted through activation of protein kinases that, in turn, phosphorylate and thereby modify the activity of critical cellular proteins [2]. cAMP promotes degradation of glycogen and trehalose and inhibits nitrogen catabolism through activation of protein kinase A [3–8]. Periodic transient variations in intracellular levels of cAMP during the yeast cell cycle have been described [9]. From analysis of *cyr*1-*cdc*35, *bcy*1, *ras*1, *ras*2, *cyr*2 and *cyr*3 mutants it has been concluded that cAMP is a positive effector for the start of the yeast cell cycle [1,10] and a negative effector for the initiation of meiosis [11]. The drastic reduction in intracellular levels of cAMP, observed under non-permissive conditions in the cell cycle mutants *cdc*35 and *cdc*25, places the cells in a state analogous to that of nutritionally deprived cells [1,13].

Here, we show that thermosensitive *cdc*35 and *cdc*25 mutations, which at the restrictive temperature decrease the level of intracellular cAMP [12,13] of *Saccharomyces cerevisiae*, also decrease the plasma membrane H^+-ATPase activity. In contrast, mitochondrial ATPase activity is increased under conditions that reduce the level of cAMP. These effects are reversed by external cAMP when *cdc*35 and *cdc*25 are associated with *cas*1, a mutation that mediates the suppression of the *cdc*35 and *cdc*25 phenotypes by external cAMP [12,13].

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2. MATERIALS AND METHODS

The haploid wild-type parental strain *S. cerevisiae* 1278b *MAT* and four isogenic *cdc* temperature-sensitive, G_1-arrested mutants were used: Le345 (*MAT*a *cdc*35-10), Be344 (*MAT*a

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*cdc*35 *cas*1 *arg*1), Gx3272 (*MAT*a *cdc*19 *arg*1), Be364 (*MAT*a *cdc*25 *cas*1 *arg*1). All *cdc* mutants, as well as the *cas*1 mutants, were isolated by the method of Boutelet et al. [12].

Strains were grown under aeration at 26°C on YEPD medium containing 1% yeast extract, 1% bactopeptone and 5.8% glucose. In some experiments the YEPD medium was supplemented with 7 mM cAMP sterilized by filtration. Cells were harvested by centrifugation at different times during exponential growth at 26°C and incubated further at 36°C in the same medium. Cells were harvested, washed with 10% glucose at 10°C and kept for 1 h on ice. The cells were then suspended in 10 ml grinding medium containing 250 mM sucrose, 50 mM imidazole-NaOH (pH 7.5). The suspension was agitated with glass beads for 2 min using a CO_2-refrigerated MSK Braun homogenizer. Crude membranes were obtained by centrifugation of the homogenate at 15000 × *g* for 40 min. The pellet, suspended in 10 mM Tris-HCl (pH 7.5) and 1 mM $MgCl_2$, was used to determine plasma membrane and mitochondrial ATPase activities. The ATPase assays at 30°C were started with 100 μg protein crude membranes in 1 ml, containing 50 mM Mes-NaOH or Tris-HCl, 6 mM NaATP, 9 mM $MgSO_4$. The mitochondrial ATPase was inhibited by 10 mM NaN_3 and the plasma membrane enzyme by 10 M vanadate, during measurements made at pH 6.0 (plasma membrane ATPase activity) and pH 9.0 (mitochondrial ATPase activity). Liberation of P_i during ATP hydrolysis was measured as described by Pullman and Penefsky [14].

The amount of protein was determined according to Lowry et al. [15], using bovine serum albumin as a standard.

The density of viable cells was estimated with a hemocytometer, after staining by methylene blue.

3. RESULTS

3.1. *Plasma membrane and mitochondrial ATPase activities of* cdc*35 mutants*

The thermosensitive mutation *cdc*35-10 modifies adenylate cyclase activity. Within 2 h after shifting from the permissive temperature of 26°C, to the restrictive temperature of 36°C, the intracellular content of cAMP decreased from 4.5 to 1.8 pmol/mg protein in the *cdc*35-10 mutant. In contrast, the cAMP content of the parental strain increased from 4 to 8 pmol/mg protein after a similar temperature shift [12]. Fig.1A,B shows the modification of plasma membrane and mitochondrial ATPase activities measured, respectively, at pH 6.0 and 9.0, in crude membranes from the wild-type *ε1278b* and the mutant *cdc*35-10 after a shift from 26 to 36°C. Within 2 h after the shift to 36°C the mutant cells continued to grow; their plasma membrane ATPase activity decreased by about half whereas the mitochondrial ATPase activity doubled. 4 h after the shift to 36°C, the cell density of mutant *cdc*35-10 reached a maximum

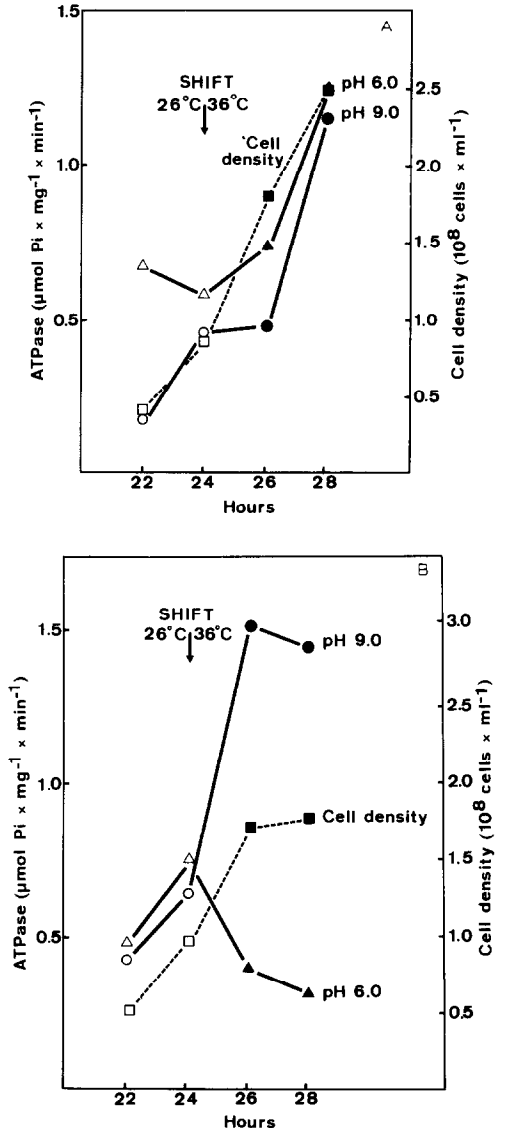

Fig.1. Time course of plasma membrane (△) and mitochondrial (○) ATPases activity of (A) wild-type ε1278b and (B) mutant strain carrying *cdc*35-10. Strains were grown exponentially on YEPD at 26°C (open symbols) and shifted to 36°C (closed symbols). ATPase activities and cell growth (□) were measured as described in the text on cells harvested at different times before and after temperature shift.

and the viable cell number began to decrease. In contrast, the cell density of the wild-type *ε1278b* tripled and plasma membrane as well as mitochondrial ATPase activities increased about 2-fold. Similar results were obtained for the double mu-

tant *Be344* which, in addition to *cdc*35, carries a *cas*1 mutation, which mediates suppression of the *cdc*35 phenotype by addition of external cAMP (fig.2). Within 1 h after the shift to 36°C, the plasma membrane ATPase activity dropped to 50% of that for cells kept at 26°C. After 4 h at 36°C, the plasma membrane ATPase was only 30% of its level at 26°C whereas that in mitochondria was at the same level at 26°C as at 36°C. The numbers of growing and viable cells were almost equal at both temperatures during the 4 h period investigated.

3.2. *Plasma membrane and mitochondrial ATPase activities in a* cdc*25 mutant*

The thermosensitive mutant *cdc*25 shows a strongly depressed cAMP pool after incubation at 36°C [13]. Regulation of adenylate cyclase activity is modified by *cdc*25 mutations [16,17]. Plasma membrane and mitochondrial ATPase activities of the *cdc*25 mutant increased almost 2-fold within 4 h of incubation at 26°C. However, at a temperature of 36°C for an equal time period, the plasma membrane ATPase activity was diminished (fig.3) to only 30% of its level at 26°C. In contrast, mitochondrial ATPase activity was greater at 36°C than at 26°C. The number of growing cells decreased appreciably after 4 h at 26°C.

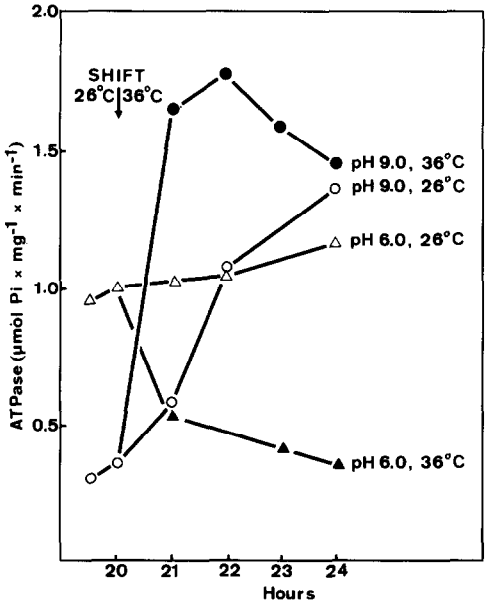

Fig.2. Time course of plasma membrane (△) and mitochondrial ATPase (○) activity at 26°C (open symbols) and 36°C (closed symbols) of mutant Be344 *cdc*35 *cas*1. The strain was grown exponentially on YEPD at 26°C; the culture was then divided into two parts: one was incubated at 26°C and the other shifted to 36°C. Both ATPase activities were measured on cells harvested at different times before and after temperature shift.

3.3. *Plasma membrane and mitochondrial ATPase activities in a* cdc*19 mutant*

In order to determine whether the observed effects on ATPase activity in *cdc*35 and *cdc*25 resulted indirectly from the block of the cell cycle in G_1 which is induced by the *cdc*35 and *cdc*25 mutations rather than from the decreased cAMP concentration, ATPase activities of another temperature-sensitive, G_1-arrested mutant, *cdc*19, defective in pyruvate kinase activity, were evaluated. In spite of the total block of growth at 36°C, the plasma membrane ATPase activity of the *cdc*19 strain Gx3272 was not modified during the 2 h period after the temperature shift. Plasma membrane ATPase activity began to decrease

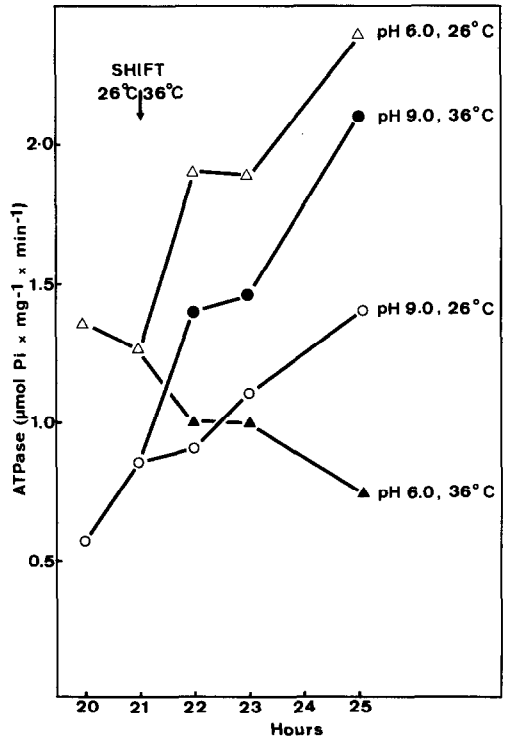

Fig.3. Time course of plasma membrane (△) and mitochondrial ATPase (○) activity at 26°C (open symbols) and 36°C (closed symbols) of mutant Be364 *cdc*25 *cas*1. Conditions as in fig.2.

slightly only after 4 h at 36°C when the cells entered a death phase (not shown). No significant changes in mitochondrial ATPase activity were observed after the temperature shift.

3.4. *Suppression by extracellular cAMP of the* cdc*35 and* cdc*25 phenotypes*

Since the original *cdc*35 and *cdc*25 strains are not permeable to external cAMP, we have used strains containing the *cas*1 mutation, which mediates phenotypic suppression of *cdc* mutants by external cAMP [12,13]. Figs 4,5 show the effects of exogenously added 7 mM cAMP on the doubly mutated strains *cdc*35 *cas*1 and *cdc*25 *cas*1

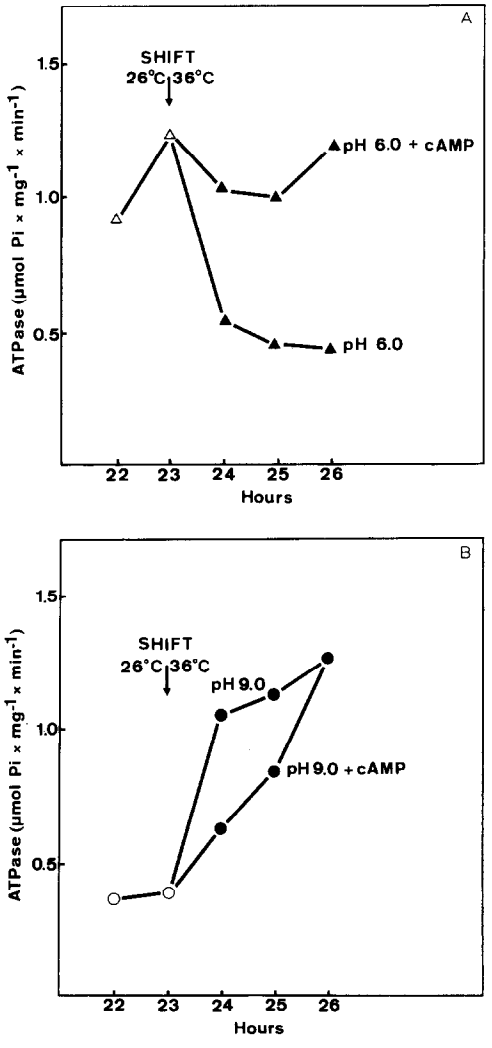

Fig.4. Time course of (A) plasma membrane (△) and (B) mitochondrial ATPase (○) activity at 36°C of mutant Be344 *cdc*35 *cas*1. The strain was grown exponentially on YEPD at 26°C (open symbols); the culture was divided into two parts and then shifted to 36°C (closed symbols). One part was supplemented with 7 mM cAMP; the other was without cAMP. Both ATPase activities were measured on cells harvested at different times before and after the temperature shift.

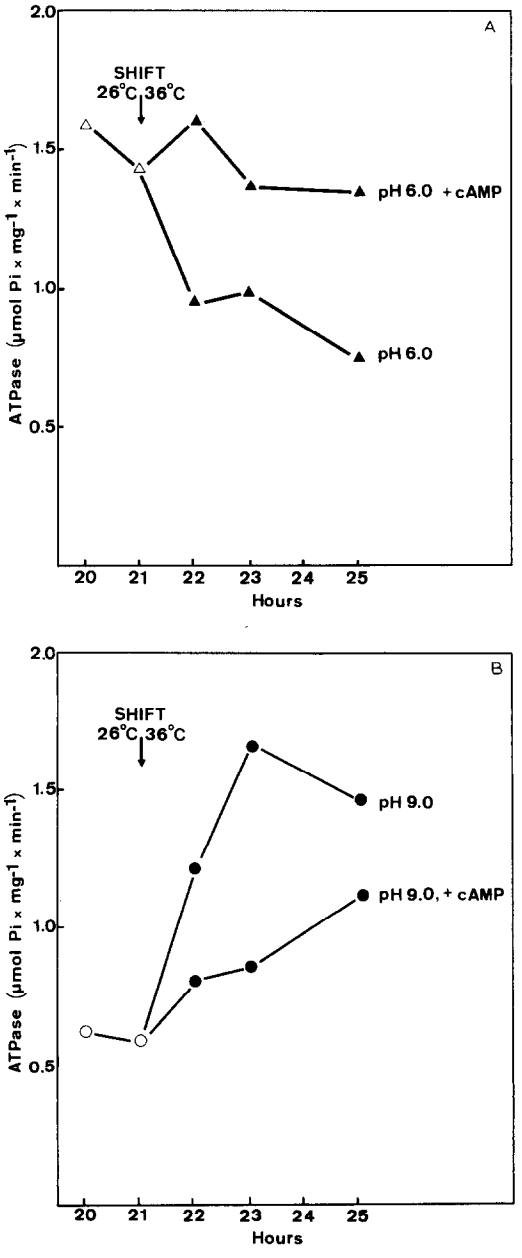

Fig.5. Time course of (A) plasma membrane (△) and (B) mitochondrial ATPase (○) activity at 36°C of mutant Be364 *cdc*25 *cas*1. Conditions as in fig.4.

incubated at restrictive temperature. In both cases we observed striking stimulation of the plasma membrane ATPase activity on addition of cAMP. In contrast, cAMP addition to *cdc*35 *cas*1 and *cdc*25 *cas*1 cultures incubated at 36°C decreased the mitochondrial ATPase activity appreciably.

4. DISCUSSION

In the presence of nutrients, the division cycle requires the expression of several *CDC* genes in order to pass the critical point called START. A drastic reduction in intracellular levels of cAMP [12,13,17] in *cdc*35 and *cdc*25 mutants shifted to non-permissive conditions leads the cells to a state analogous to that of nutritionally deprived, unbudded and non-growing cells. Adenylate cyclase encoded by the *CDC*35 gene is positively regulated by the *CDC*25 gene product [16,17]. The cAMP system to which *CDC*25 and *CDC*35 belong appears to play a direct role in the molecular switch between proliferation and differentiation, by promoting the phosphorylation of target proteins which control the switch [6].

We believe that the plasma membrane H^+-ATPase might be such a target for the cAMP cascade. Indeed, in 1975, we reported that in the yeast *Schizosaccharomyces pombe*, the uptake of amino acids, sugars and nucleosides by cells placed under nutritional down-shift conditions (e.g. limiting carbon or nitrogen supply) was markedly stimulated by the addition of external cAMP [18]. Because extracellular acidification by yeast cells on glucose addition was also stimulated by cAMP addition and because the glucose-induced acid efflux was abolished by the ATPase inhibitor Dio-9, we proposed that yeast plasma membranes contained an H^+-ATPase activity which drives cell H^+ efflux as well as the active uptake of nutrients and which is controlled by cAMP. To date it has not been possible to check further this latter point because similar responses were not obtained in the yeast *S. cerevisiae*. However, recently, a connection between cAMP metabolism and plasma membrane ATPase activity in *S. cerevisiae* was demonstrated by Portillo and Mazon [19] who observed that a *cdc*25 mutant had lost the activation by glucose of the H^+-ATPase activity first described for a wild type by Serrano [20]. The plasma membrane from *S. cerevisiae* has been shown recently to contain a seryl-protein kinase which phosphorylates the H^+-ATPase as well as other membrane components [21]. This kinase activity was not autophosphorylated and was cAMP-independent, therefore seeming to differ from the cAMP-dependent protein kinase activity with an associated regulatory subunit identified recently in *S. cerevisiae* plasma membranes by Behrens and Mazon [22].

We have shown here that the *cdc*35 and *cdc*25 strains, where the intracellular level of cAMP is reduced by a shift to non-permissive conditions, exhibit reduced plasma membrane ATPase activity. The suppression by extracellular cAMP of these phenotypes in the double mutants *cdc*35 *cas*1 and *cdc*25 *cas*1 further supports our conclusion that in *S. cerevisiae*, cAMP exerts a positive control on the plasma membrane H^+-ATPase activity, most probably through the cAMP-dependent kinase. That this control is not an indirect consequence of the arrest in G_1 of the cell cycle or modification of ATP production is demonstrated by the observation that the thermosensitive *cdc*19 mutant which is also blocked in G_1, as a result of deficient pyruvate kinase, does not show modifications of plasma membrane ATPase activity.

We also report transient stimulation of the specific mitochondrial ATPase activity in *cdc*35-10 and *cdc*25 mutants placed at the restrictive temperature which reduces the plasma membrane ATPase activity. Inhibition of mitochondrial ATPase activity is obtained by the addition of cAMP to *cdc*35 and *cdc*25 mutants made sensitive to external cAMP by the *cas*1 mutation. This observation suggests that cAMP is a negative effector for the mitochondrial ATPase activity. This conclusion lends independent support to a recent proposal of the control of mitochondrial metabolism by cAMP put forward by Müller and Bandlow [23] who identified a cAMP-dependent protein kinase in yeast mitochondria.

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