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cAMP Promotes the Synthesis in Early G_1 of gp115, a Yeast Glycoprotein Containing Glycosyl-phosphatidylinositol*

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Rita Grandori, Laura Popolo, Marina Vai, and Lilia Alberghina‡

From Sezione di Biochimica Comparata, Dipartimento di Fisiologia e Biochimica Generali, Via Celoria 26, 20133 Milano, Italy

The glycoprotein gp115 (M_r = 115,000, pI 4.8–5) is localized in the plasma membrane of *Saccharomyces cerevisiae* cells and maximally expressed during G_1 phase. To gain insight on the mechanism regulating its synthesis, we have examined various conditions of cell proliferation arrest. We used pulse-labeling experiments with [^{35}S]methionine and two-dimensional gel electrophoresis analysis, which allow the detection of the well characterized 100-kDa precursor of gp115 (p100). In the cAMP-requiring mutant *cyr1*, p100 synthesis is active during exponential growth, shut off by cAMP removal, and induced when growth is restored by cAMP readdition. The inhibition of p100 synthesis also occurs in TS1 mutant cells (*ras1ras2-ts1*) shifted from 24 to 37 °C. During nitrogen starvation of *rca1* cells, a mutant permeable to cAMP, p100 synthesis is also inhibited. cAMP complements the effect of ammonium deprivation, promoting p100 synthesis, even when added to cells which have already entered G_0. Experiments with the *bcy1* and *cyr1bcy1* mutants have indicated the involvement of the cAMP-dependent protein kinases in the control of p100 synthesis. Moreover, the synthesis of p100 was unaffected in A364A cells, terminally arrested at START B by α-factor. These results indicate that the switch operating on p100 synthesis is localized in early G_1 (START A) and is one of the multiple events controlled by the cAMP pathway.

In the yeast *Saccharomyces cerevisiae* the commitment to initiate a new division cycle occurs in the G_1 phase at the level of the regulatory area called START (1). Information coming from the environment and from inside the cell is integrated at START. At this stage the cell decides to progress in a new cycle or to undergo alternative developmental pathways such as sporulation, mating or entry into the resting phase (G_0). The deprivation of essential nutrients (nitrogen, sulfur, etc.) causes the entrance in the resting state, which is similar to the G_0 arrest of mammalian cells (2).

START can be divided into two phases: A and B (3). The block at the START A, determined by mutations in Class II genes (CDC19, CDC25, CDC33, CDC35), inhibits cell cycle progression at a stage in which cells are not competent for mating, causing a reduction of RNA synthesis and therefore of growth, similarly to entry into G_0. Mutations of the class I genes (CDC28, CDC36, CDC37, CDC39) cause an arrest at START B: inhibition of cell division occurs, but cells continue to grow (RNA and protein synthesis are not affected for some time) and they are competent to mate (4).

Extensive genetic and biochemical analysis provided evidence on the involvement in the START regulatory events of the cyclic AMP (cAMP) metabolic pathway and of its intracellular transducer, the cAMP-dependent protein kinases (reviewed in Ref. 5). The initial indication that cAMP is a positive effector of yeast cell proliferation came from the observation that the removal of cAMP from the cAMP-requiring *cyr1* mutant, causes a START A arrest (6). The *bcy1* mutation bypasses this defect (6). CYR1, which is allelic to CDC35, is the structural gene of adenylate cyclase (7), while BCY1 codes for the regulatory subunit of cAMP-dependent protein kinases (8, 9). Following studies have shown that the RAS1 and RAS2 proteins of yeast are modulators of adenylate cyclase and their structural and functional interaction with the CDC25 gene product has been postulated (reviewed in Ref. 10). Recent evidence indicates that CDC25 gene product is located upstream to RAS proteins, probably acting on them as an exchange GDP/GTP factor dependent on nutritional signals (10). Thus, in yeast the signal transduction pathway for growth is mediated by the RAS-cAMP pathway and the resulting modulation of cAMP levels regulates cell cycle progression through early G_1 (1, 11). cAMP evokes many pleiotropic effects in yeast cells (12–14) including the modulation of the synthesis of specific proteins (15–17). The characterization of these cellular products could prove to be interesting for the comprehension of the physiological effects induced by this signal. The synthesis of the heat-shock proteins ubiquitin and p118 was shown to be negatively controlled by cAMP and a possible role of these proteins in proliferation arrest has been suggested (15, 16). In the present paper we have investigated the modulation of the expression of gp115, a yeast glycoprotein of M_r 115,000 and pI 4.8–5 containing glycosyl-phosphatidylinositol as a plasma membrane anchor domain (18–20). In previous papers it has been reported that its 100-kDa precursor (p100) is maximally expressed in the G_1 phase (21, 22). Post-translational processing studies have shown that p100 is the core-glycosylated form of gp115 detectable by short-labeling experiments (18–22). Therefore, p100 precursor level was used in the present study as a probe for the gp115 biosynthetic rate. Here we report that the synthesis of p100 is positively regulated by cAMP through the activity of cAMP-dependent protein kinases. Moreover, the synthesis of p100 is unaffected by cell cycle arrest at START B. The reported findings indicate that the switch operating on p100 synthesis is specifically controlled by the mechanism regulating the cell cycle at START A.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—The following *Saccharomyces cerevisiae* strains were used in the present study: OL214 (a/α cdc25/

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‡ To whom correspondence should be addressed.

+ +/cdc35-10 ade1/+ +/leu2 +/his7 rca1/rca1) obtained from M. Jaquet (Laboratoire d'Information Génétique et Developpement, Orsay Cedex, France) and AM7-11D (a cyr1), AM711DR-4 (a cyr1 bcy1), AM9-8B (α bcy1) obtained from K. Matsumoto (DNAX Research Institute, Palo Alto, CA). *S. cerevisiae* strain A364A (a ade1 ade2 ura1 his7 lys2 tyr1 gal1) and the temperature-sensitive strain derived from A364A carrying the allele cdc25-1 (strain 321) were obtained from the Yeast Genetics Stock Center (Berkeley, CA). The strain TS1 (α ade2 can1-100 his3 leu2-3,112 lys1-1 ura352 ras1::URA3 ras2ts1::SUP16) and the parental one 19D1 were supplied by O. Fasano (European Molecular Biology Laboratory, Heidelberg, FRG). The growth medium was made up with 6.7 g/liter of Difco yeast nitrogen base (YNB) without amino acids, 2% glucose, and with the appropriate supplements as previously described (23). For nitrogen-free medium (YNB-N), $(NH_4)_2SO_4$ was omitted. Cells were grown in batch cultures in a shaking water bath at 30 °C. Temperature-sensitive strains were grown at 24 °C (permissive temperature) and transferred during exponential growth to 37 °C (restrictive temperature). Cyclic AMP (Sigma) was added to a final concentration of 3 mM. α-Factor from Bachem (Basel, Switzerland) was resuspended in 10 mM HCl, 0.1 mM EDTA, and 1 mM β-mercaptoethanol (0.3 mM stock solution).

Determination of Cell Number, Cell Volume Distribution, and Percentage of Budded Cells—Growth was monitored as increase in cell number determined using a Coulter ZB1 counter (Counter Electronics, Harpenden, UK) as described previously (23). The cell volume distributions were obtained using a Coulter Channalyzer C-1000. The percentage of budded cells was determined by direct microscopic counting of at least 400 cells that had been fixed in formalin and mildly sonicated.

Labeling Conditions and Sample Preparation—The pulse-labeling experiments were performed during exponential growth or during different types of cell cycle arrest at a cell density ranging from 3×10^6/ml to about 7×10^6/ml. Subcultures (5–20 ml) were labeled with [^{35}S]methionine (10 μCi/ml) for 8 min and at the end of the pulse cells were quickly collected by filtration, and extracts were prepared as described previously (21). About $2–3 \times 10^6$ dpm were subjected to first dimension separation.

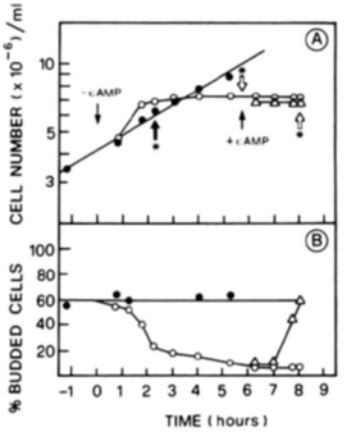
Two-dimensional Gel Electrophoresis—Protein extracts were resolved according to a procedure routinely used in our laboratory (21) slightly modified from O'Farrell (24). After the second dimension run gels were fixed, treated with Enlighting (New England Nuclear, Dreiech, FRG), and then dried.

cAMP Assay—Yeast cells ($2–4 \times 10^8$) were collected by filtration and quickly washed with a small volume of ice-cold water. Cells were resuspended in 1 M acetic acid and frozen at −80 °C. Following 3 cycles of freeze-thawing, the extracts were centrifuged. The supernatant was lyophilized. The pellet was resuspended in 1.5–2 ml of 1 N NaOH and processed for protein determination using the bicinchoninic acid assay kit (Pierce Chemical Co.). The lyophilized supernatant was resuspended in 200–300 μl of 0.05 M Tris, 4 mM EDTA, pH 7.5, and cAMP was determined according to the instructions of the Amersham Corp. cAMP assay kit.

RESULTS

Gp115 Synthesis Is Inhibited in Conditions of Reduced cAMP Level and Cell Cycle Arrest—To analyze a condition of drastic reduction of intracellular cAMP level, we used the *cyr1* mutant (strain AM7-11D), deficient in adenylate cyclase activity (6). This strain requires exogenous cAMP in order to grow (6). In the experiment reported in Fig. 1, *cyr1* mutant cells were grown in minimal medium supplemented with 0.5 mM cAMP. In this condition cells grew exponentially with a duplication time of about 4.3 h (Fig. 1*A*). The shift to a cAMP-free medium resulted in growth inhibition, accompanied by a rapid decrease of the percentage of budded cells (Fig. 1, *A* and *B*). The overall rate of protein synthesis was severely inhibited 5 h after the shift (15% residual rate, data not shown). To evaluate the gp115 expression, we used short pulses of [^{35}S]methionine (8 min) followed by separation of labeled proteins with high resolution two-dimensional gel electrophoresis. In this way, only the intermediate precursor p100 is detected since the processing of gp115 is a rather slow process requiring about 15–20 min (18, 19). This protocol was used to evaluate the synthesis of p100 in all the experiments described herein. In the *cyr1* mutant 5.5 h after cAMP removal, a drastic inhibition of p100 synthesis was observed (Fig. 1*D*) with respect to the exponentially growing culture (Fig. 1*C*). This response was reversed by cAMP readdition. In fact, the percentage of budded cells rapidly increased (Fig. 1*B*) and the induction of high levels of p100 synthesis was observed after cAMP readdition (Fig. 1*E*), most likely enhanced by the high degree of synchronization of the cells, as the budding profile suggests.

As shown in Fig. 1 our two-dimensional gels allow also the resolution in a well recognizable area, of an acidic, high M_r polypeptide that, on the basis of its characteristics and typical aspect of migration, has been identified as the protein p118 (M_r = 118,000, pI A form = 4.2, and B form = 4.3). The synthesis of p118 has been reported to be induced by heat shock, starvation conditions (25), and to be negatively regulated by cAMP (15). In the experiment reported in Fig. 1 the synthesis of p118 (A and B isoforms) is induced by cAMP



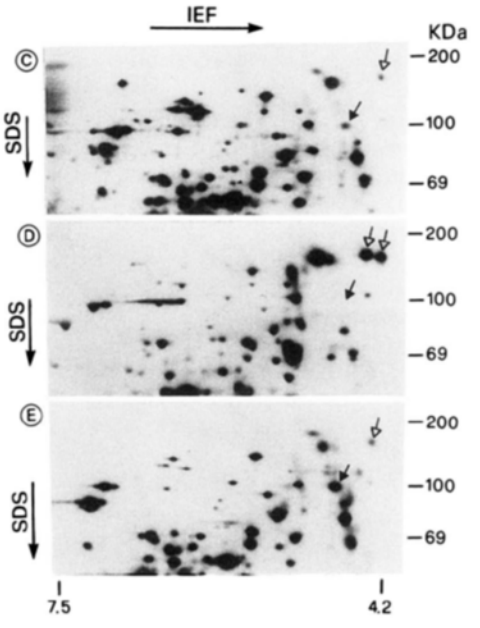


FIG. 1. **Expression of p100 in the *cyr1* mutant.** *cyr1* mutant cells (strain AM7-11D) were grown in the presence of 0.5 mM cAMP. An aliquot of the culture was filtered and resuspended in cAMP-free medium at time 0. At the times indicated by the *thin arrows* in *A*, cAMP was removed or added back to the culture. Cell number (*A*) and percentage of budded cells (*B*) during growth in the presence of cAMP (-●-), after removal (-○-) and readdition of cAMP (-△-). Cells were pulse-labeled with [^{35}S]methionine for 8 min at the times indicated by the *asterisks* in *A* and protein analyzed by two dimensional gel electrophoresis. The pH range is from 7.5 to 4.2. *C*, control culture with cAMP present; *D*, culture deprived of cAMP; *E*, the same culture 130 min after cAMP readdition. The *closed* and *open arrows* indicate p100 and p118, respectively. *SDS*, sodium dodecyl sulfate; *IEF*, isoelectric focusing.

depletion and reduced by cAMP readdition. The identity of behavior in the regulatory response observed, gives more confidence to the identification of p118 in our two-dimensional gels in which its mobility is slightly slower than the reported one (15). The residual synthesis of p118 in cycling *cyr1* cells (Fig. 1*C*), usually undetectable in cycling cells, was observed also in the cyr1-2 ts mutant (15) and it can be attributed to the elongation of the unbudded phase in these cells.

If cAMP has a role in gp115 expression, other mutations involved in the cAMP biosynthetic pathway should also affect its synthesis. We therefore analyzed the behavior of the protein synthesis pattern in the TS1 mutant, which contains a disrupted RAS1 gene and a temperature-sensitive RAS2 allele (26). At 37 °C (restrictive temperature) adenylate cyclase activity is reduced due to the lack of functional RAS proteins (26). The synthesis of p100 was analyzed by pulse-labeling TS1 cells exponentially growing at 24 °C or 2.5 h after a shift to 37 °C. Following the shift, cells prevalently accumulated in the unbudded phase (85% unbudded cells after 2.5 h) in agreement with results obtained by De Vendittis *et al.* (26). As reported in Fig. 2*B*, the temperature shift results in the suppression of p100 synthesis confirming that the expression of this protein is also modulated by the presence of the RAS2 product. Moreover, the synthesis of p118 is once again modulated in an opposite way with respect to p100 being greatly stimulated at 37 °C in TS1 cells. The synthesis of a set of acidic proteins (indicated in Fig. 2*B*), close to p118 and normally barely detectable, is substantially increased. These proteins are poorly characterized: they were described as G_1-specific proteins by Lorincz (22) and shown to be induced by mild heat shock by Verma *et al.* (25).

Finally, to avoid any possible side effect of the temperature on the evaluation of p100 synthesis, the isogenic strain (19D1) of TS1 was labeled both at 24 °C and 37 °C according to the protocol previously described. No significant variation on the synthesis of this protein was observed (data not shown). This is also in accordance with the kinetics of response of p100 synthesis to a mild heat shock; in fact, after an initial inhibition, p100 expression returns to pre-heat-shock levels within 60 min from the temperature up-shift (25).

cAMP Prevents the Inhibition of gp115 Synthesis upon Nitrogen Starvation—We assayed gp115 synthesis during a cell cycle arrest induced by nitrogen starvation. For this purpose we used the *rca1* mutant (strain OL214), isolated by Boy-Marcotte *et al.* (27), since its properties allowed us to also test the effects of exogenously added cAMP. When exponentially growing *rca1* cells are transferred to nitrogen-free medium cell growth, monitored as increase in cell number, proceeds unaffected for about 3 h and then ceases (Fig. 3*A*). The percentage of budded cells progressively decreases, as shown in Fig. 3*B*, going from a value of approximately 50% to about 4%. After 3–4 h from the shift cells are uniformly arrested in the unbudded phase. When *rca1* cells are shifted to nitrogen-free medium containing 3 mM cAMP, the cell number increase is reduced, and the percentage of budded cells gradually declines until stabilizing at a value around 30%. A previous report has shown that in this condition cells are arrested at different stages of the cell division cycle and do not acquire phenotypical characteristics of starving cells such as the resistance to zymolase and to heat treatment (27). In order to check the specificity of cAMP effects on *rca1* cells, a separate culture was shifted to nitrogen-free medium containing 5′-AMP. No variation on the kinetics of entry into stationary phase and on budding has been found with respect to the untreated culture (data not shown).

Moreover, the analysis of cell volume distributions shows

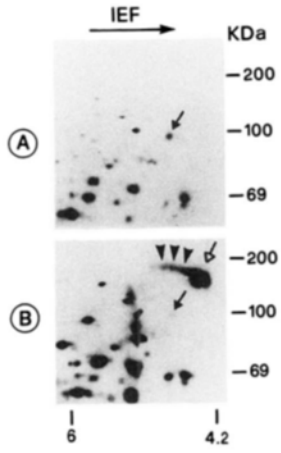


FIG. 2. **Analysis of p100 synthesis in the TS1 mutant.** Exponentially growing TS1 mutant cells at 24 °C (*A*) were shifted at a cell density of 5×10^6 cells/ml to 37 °C (*B*). After 2.5 h cells were pulse-labeled with [^{35}S]methionine. The control culture at 24 °C was pulse-labeled at the moment of the shift. The *closed* and *open arrows* indicate p100 and p118. The *small arrowheads* indicate a group of G_1-specific proteins (22). pH range from about 6 to 4.2 is shown in this and in the following figures. *IEF*, isoelectric focusing.

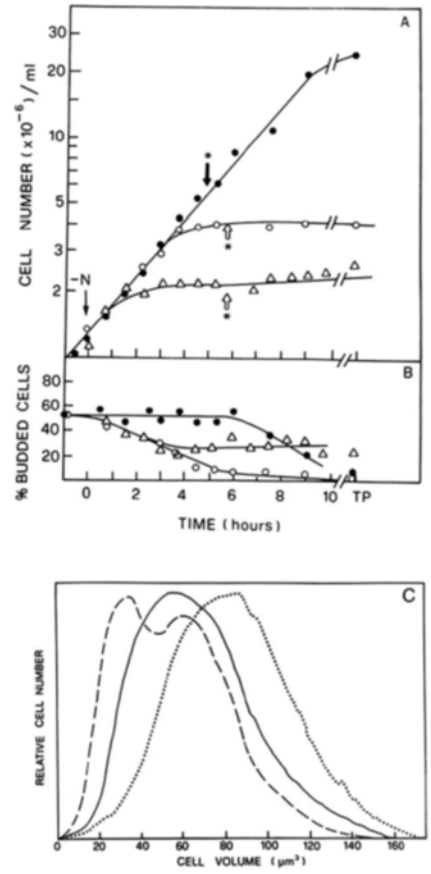


FIG. 3. **Effect of addition of cAMP on entry in G_0.** Exponentially growing diploid *rca1* cells (strain OL214) were filtered at zero time and cells were transferred to nitrogen-free medium. The cell number (*A*) and the percentage of budded cells (*B*) of exponentially growing cells (–●–), or cells transferred to starving medium without (–○–) or with 3 mM cAMP (–△–) were determined at various time intervals. The cell volume distributions (*C*) were acquired at 5.5 h from the shift; ——, exponential growth; – – –, nitrogen-starved cells; and. . . . cAMP-treated cells. The *asterisks* in *A* indicate the moment in which cells were pulse-labeled. *TP*, terminal phenotype.

an enlargement of cAMP-treated cells with respect to both nitrogen-starved and exponentially growing cells (Fig. 3*C*). This further effect of cAMP on cell size has been interpreted in the framework of a yeast cell cycle model developed in our laboratory (28).

Starved and cAMP-treated *rca1* cells were pulse-labeled with [^{35}S]methionine at the times indicated in Fig. 3*A*. The synthesis of p100 was shut off (and that of p118 induced) about 5.5 h after the shift of exponentially growing *rca1* cells to starvation medium (Fig. 4, *A* and *B*). Several experiments have shown that a 5-h incubation in nitrogen-free medium is sufficient to acquire this specific phenotype. A similar effect of reduction of p100 synthesis was also found using the A364A strain (data not shown). When 3 mM cAMP is present from the moment of the shift of *rca1* cells to nitrogen-free medium we have observed the persistence of p100 synthesis and prevention of p118 induction as shown in Fig. 4*C*. We have also examined the synthesis of some acidic high molecular weight heat-shock proteins typical of G_0 arrest (2, 29) and they were found to undergo the same modulation of p118.

Although cAMP complements the effects of nitrogen starvation on p100 synthesis, no significant variation in cAMP levels between exponentially growing and starved *rca1* cells was observed (Table I).

Gp115 Synthesis Is Not Inhibited by Cell Cycle Arrests That Do Not Block Cell Growth—So far we have examined conditions which define a type of arrest at START A (G_0 arrest). To understand whether such a specific type of arrest is required, or if p100 synthesis is affected by any other condition preventing mitotic cycle, we also analyzed cell cycle blocks at a later stage (START B). Haploid A364A cells were treated with 1 μM α-factor. Following this treatment, the percentage of budded cells rapidly decreased till reaching 10% in less than 3 h and cells arrested with a presynthetic content of DNA (30). Blocked cells were pulse-labeled with [^{35}S]methionine and the protein pattern was compared to that of exponentially growing cells. The synthesis of p100 in arrested cells is similar to that of control ones (Fig. 5). In this condition the intracellular cAMP levels were measured and they were not significantly different from those of cycling cells (Table I).

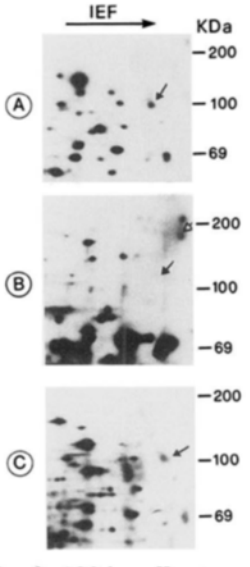


FIG. 4. **Synthesis of p100 in cells starved in the presence of cAMP.** The two-dimensional patterns of exponentially growing *rca1* cells before (*A*) and after transfer to nitrogen-free medium in the absence (*B*) or presence of 3 mM cAMP (*C*) were obtained after pulse-labeling the cells at the times indicated by the *asterisks* in Fig. 3. The *closed* and *open arrows* indicate p100 and p118, respectively. *IEF*, isoelectric focusing.

TABLE I

Intracellular cAMP levels during nitrogen starvation and α-factor treatment

Strain	Culture condition[a]	% Budded cells	cAMP[b]	
			pmol/mg protein	*pmol/10^{-7} cells*
OL214	E	63	10.9	1.3
	−N 5 h	4	11.5	1.0
	−N 21 h	2	12.4	0.9
	−N 71 h	2	12.7	1.1
A364A	E	60	8.5	0.70
	+αF 1.5 h	27	9.5	0.75
	+αF 2.5 h	10	11.4	0.77
	+αF 3.5 h	6	8.5	0.68

[a] OL214 cells (*rca1/rca1*) exponentially growing (E) on minimal medium (YNB-glucose) were shifted at a cell density of 2×10^6 cells/ml to the same medium deprived of ammonium (−N). A364A cells exponentially growing on minimal medium were treated, at a cell density of 3×10^6 cells/ml, with 1 μM α-factor (αF). At the times indicated aliquots of the cultures were withdrawn for cAMP assay.

[b] cAMP and protein were determined as described under "Experimental Procedures."

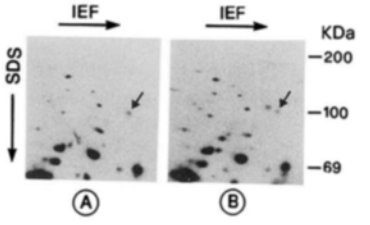


FIG. 5. **Effect of α-factor treatment on p100 expression.** Exponentially growing cells (strain A364A) were treated with 1 μM α factor at a cell density of 3×10^6 cells/ml. After 3 h control cells (*A*) and pheromone-treated cells (*B*) were pulse-labeled with [^{35}S]methionine for two-dimensional gel electrophoresis. The *closed arrow* indicates p100. *IEF*, isoelectric focusing; *SDS*, sodium dodecyl sulfate.

This experiment indicates that inhibition of mitotic cycle is not in itself sufficient to suppress p100 synthesis.

Regulation of gp115 Synthesis by cAMP Is Likely to Be Mediated by cAMP-dependent Protein Kinases—In yeast cells cAMP acts through cAMP-dependent protein kinases which carry out several of its pleiotropic effects by phosphorylating specific target proteins. To understand if p100 synthesis is modulated by cAMP through the kinase activity, the double mutant *cyr1bcy1* was analyzed. In this strain (AM7-11DR-4) a constitutive activity of the kinases complements the *cyr1* mutation allowing cells to also grow in the absence of cAMP (6). As shown in Fig. 6*A* the double mutant expresses p100.

The effect of the *bcy1* mutation on the synthesis of p100 was also evaluated during nitrogen-starvation. Mutant (*bcy1*) cells are unable to arrest in G_0 phase and progressively lack vitality in starving conditions (31–32). To determine if the constitutive activity of protein kinases prevents the typical repression of p100 synthesis during nitrogen starvation, we pulse-labeled *bcy1* cells either during exponential growth or after 5.5 h from a shift to nitrogen-free medium (Fig. 6, *B* and *C*). At this time the parental strain AM3-4B had already entered G_0 state (data not shown). During the starvation, *bcy1* cells show a constitutive synthesis of p100 (Fig. 6*C*). Unexpectedly, the inhibitory effect of *bcy1* mutation on p118 synthesis (15) is more effective during starvation than in exponential growth.

In both the experiments in Fig. 6, *A* and *B*, a residual synthesis of p118 was observed during exponential growth apparently in contrast with previous results and published

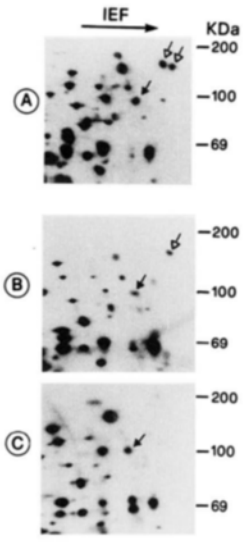


FIG. 6. **Analysis of p100 synthesis in *cyr1bcy1* and *bcy1* mutant cells.** Cultures of *cyr1bcy1* cells (*A*) were pulse-labeled during exponential growth. *bcy1* cells were pulse-labeled during exponential growth (*B*) and 5.5 h after a shift to nitrogen-free medium (*C*). The *closed* and *open arrows* indicate p100 and p118. *IEF*, isoelectric focusing.

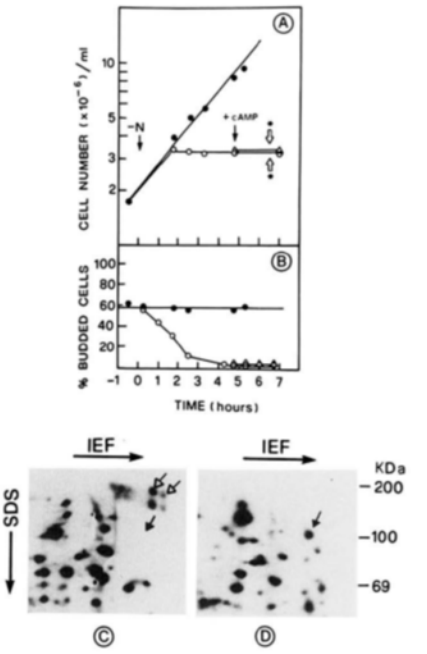


FIG. 7. **Effect of cAMP added to starved cells on p100 synthesis.** Cell number (*A*) and the percentage of budded cells (*B*) of an exponentially growing culture (–●–) and another transferred at zero time to nitrogen-free medium (–○–) to which 3 mM cAMP was added 5 h later (–△–). Cells were pulse-labeled with [^{35}S]methionine in the presence or absence of cAMP at the times indicated by the *asterisks* in *A*. *C*, starved cells; *D*, starved cells treated for 1.5 h with cAMP. The *closed* and *open arrows* indicate p100 and p118. *SDS*, sodium dodecyl sulfate; *IEF*, isoelectric focusing.

data (15). Since the parental strain AM3-4B in exponential growth also shows the presence of p118 (data not shown) we believe that the genetic background of these strains affects the basal level of p118 synthesis.

cAMP-dependent Regulation of p100 Synthesis Is Uncoupled from Cell Growth—We tested the effects of cAMP addition to *rca1* cells after 5 h of nitrogen starvation. At this time cells showed the characteristic phenotype of starved cells as already reported in Fig. 3, a complete suppression of p100 synthesis and the induction of p118 expression as already reported in Fig. 4*B*. The addition of 3 mM cAMP to these cells has no effect on the cell number, the percentage of budded cells (Fig. 7, *A* and *B*) and on the bimodal distribution of cell volumes previously shown in Fig. 3*C* (data not shown). However, the addition of cAMP brings about significant changes in the protein synthesis pattern. As shown in Fig. 7*C* and *D*, p100, and p118 synthesis reverts to the levels characteristic of exponential growth: p100 synthesis is greatly stimulated whereas p118 is suppressed. This experimental condition is of particular interest because it only allows the primary responses to cAMP to be detected, uncoupling them from cell growth induction. The results obtained suggests that cAMP plays a direct regulatory role in the synthesis of p100 and, as far as p118 is concerned, they are in agreement with previously reported data (15).

DISCUSSION

In previous papers we have identified a yeast protein (p100) whose synthesis is repressed in the START mutant cdc25 blocked at 37 °C and induced during the release from cell cycle arrest (21). Furthermore, Lorincz *et al.* (22) have identified this polypeptide as a member of a group of proteins whose synthesis is periodic with a maximum of expression in the late G_1 phase. Moreover, p100 has been found to be a precursor form of the stable *N*-glycosylated protein gp115 (18, 19). Through a detailed characterization of gp115 biosynthetic pathway we have shown that p100 contains glycosyl-phosphatidylinositol and six core-oligosaccharide chains which are further processed to yield the mature gp115 (18–20). The transfer of the core-glycosylated precursor from endoplasmic reticulum to Golgi appears to be the limiting step of gp115 biosynthesis (19).

In this paper we have investigated whether the synthesis of gp115, determined by monitoring the appearance of its precursor p100, is modulated by the signal transduction pathway which involves cAMP. In the first part of our work we have pointed out that a reduction of cAMP levels is correlated to the inhibition of p100 synthesis. This has been achieved by using both the *cyr1* mutant, in which the activity of adenylate cyclase is abolished by a mutation in the structural gene (6–7), and the TS1 mutant, which lacks RAS1 protein and carries a ts mutation in the RAS2 gene (26). Both these mutant genotypes induce phenotypical pleiotropic responses among which we have identified the complete inhibition of p100 synthesis. In these experiments we have pulse-labeled the cells as soon as the typical parameters of arrested culture were stabilized. At this time p100 synthesis has already attained a complete inhibition. Our analysis also allows the identification of the heat-shock glycoprotein p118 (15) which behaves in an opposite way to p100, because it is induced by reduction of cAMP levels, in agreement with reported data (25).

The effect of nitrogen starvation on p100 synthesis was also evaluated. Deprivation of this essential nutrient induces arrest in early G_1 (G_0 arrest). The synthesis of p100 is repressed in nitrogen-starved cells, while it remains active when cAMP is supplemented during starvation (Fig. 4). Our result is in accordance with data showing that the onset of resting state in *rca1* cells is antagonized by cAMP (27) and extends the analysis to the changes in the two-dimensional pattern of acidic high molecular weight proteins. It is noteworthy that the protein pattern has been found to respond to cAMP even when the latter was added to cells that had already entered the resting state. The addition of cAMP to cells arrested by a

5-h incubation in nitrogen-free medium drastically induces the synthesis of p100 and blocks the expression of p118 (Fig. 7). The important feature of this experiment is that the effect on specific protein expression is uncoupled from the secondary physiological effects on cell mass accumulation and on budding, typically observed in the other conditions of cAMP stimulation. This result clearly indicates that the synthesis of p100 is a direct response to regulatory events evoked by cAMP and we can therefore rule out that it is merely a consequence of growth stimulation. Finally, preliminary results on the kinetics of p100 synthesis induction indicate that the activation of p100 synthesis is a rather early event which is detectable 15 min after cAMP addition (data not shown).

The reduction of p100 synthesis (and induction of p118) had been reported during sulfur starvation (25). These two conditions of deprivation of essential nutrients appear to differ in their effects on cAMP levels. While sulfur starvation has been reported to cause a reduction of cAMP levels (33), during nitrogen deprivation of *rca1* cells we could not measure variations of cAMP level between starved cells and exponentially growing cells (Table I). This could be due to difficulties in comparing cAMP levels between blocked and proliferating cells. In fact, cAMP levels fluctuate along the cell cycle (34) so that the measurement in growing cells refers only to an average value. Alternatively, it can be postulated that ammonium ions act through a signal transduction pathway different from that of cAMP, however, hypostatic to it. Studies on the similarities in the phosphoprotein patterns between nitrogen-starved cells and cyr1-2(ts) mutant cells at 37 °C tend to exclude this hypothesis so far (35).

The possible involvement of cAMP-dependent protein kinases in the modulation of p100 synthesis was investigated using the double mutant *cyr1bcy1*, in which the defect of growth, due to the absence of a functional adenylate cyclase, is suppressed by the constitutive activity of cAMP-dependent protein kinases. In this mutant *bcy1* mutation restores p100 synthesis complementing the inhibitory effect of *cyr1* mutation. Moreover, the synthesis of p100 appears unaffected by transferring *bcy1* cells to nitrogen-free medium. Thus, in both conditions of adenylate cyclase defect and nitrogen starvation, the addition of cAMP or the activation of cAMP-dependent protein kinases brings about the same effect on p100 synthesis. These results strongly indicate that cAMP-dependent phosphorylation of target proteins regulates the expression of p100. We do not know yet whether this mechanism involves a translational or a transcriptional control.

Finally, the analysis of α-factor-treated cells has indicated that p100 does not respond to the mechanism of cell cycle regulation operating at START B. The lack of sensitivity of p100 synthesis to cdc28 mutation (data not shown) also supports this conclusion. Moreover, we found that cAMP level was not perturbed by α-factor addition (Table I). Although this determination is subjected to the above mentioned uncertainties, other data support the hypothesis that the block at phase B of START by pheromone does not involve the cAMP pathway (36). Moreover, this experiment provides further elements supporting that p100 synthesis is correlated to cAMP levels and not directly to cell cycle progression.

In conclusion, our results indicate that the switch that regulates p100 synthesis is one of the targets of the mechanism controlling the cell cycle at START A: it is turned off in conditions of cell cycle arrest, caused by a deficiency in cAMP production or by nitrogen starvation. In this regulatory area cAMP is sufficient to promote the p100 synthesis by a cAMP-dependent protein kinase-mediated mechanism.

Although several examples of yeast proteins modulated by the cell cycle have been reported, the molecular mechanisms of this regulation are still poorly characterized. Our work provides a preliminary evidence that cAMP is one of the possible factors mediating cell cycle modulation of a yeast-specific cellular product.

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