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Regulation of Phospholipid Biosynthesis in *Saccharomyces cerevisiae* by Cyclic AMP-Dependent Protein Kinase†

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The addition of cyclic AMP (cAMP) to *Saccharomyces cerevisiae cyr1* mutant cells resulted in an increase in the rate of phosphatidylinositol synthesis at the expense of phosphatidylserine synthesis. The decrease in phosphatidylserine synthesis correlated with the down regulation of phosphatidylserine synthase activity by cAMP-dependent protein kinase phosphorylation. The increase in phosphatidylinositol synthesis was not due to the regulation of phosphatidylinositol synthase by cAMP-dependent protein kinase.

Phosphatidylserine (PS) synthase plays a major role in the overall regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae* (16, 17, 21). PS synthase is regulated by phosphorylation via cyclic AMP (cAMP)-dependent protein kinase (13). The phosphorylation of the PS synthase 23,000-M_r subunit results in a 60 to 70% reduction in PS synthase activity (13). Immunoprecipitation of the phosphorylated and dephosphorylated forms of the PS synthase 23,000-M_r subunit from a *bcy1* mutant (which has high cAMP-dependent protein kinase activity) and a *cyr1* mutant (which has low cAMP-dependent protein kinase activity) with corresponding reduced and elevated levels of PS synthase activity, respectively, confirmed that the enzyme is regulated by phosphorylation in vivo (13). In the present study we showed that the levels of cAMP and thus of cAMP-dependent protein kinase activity regulated the rates of phospholipid biosynthesis in vivo.

Our studies were facilitated by the use of strain AM18-5C (*MATα ade6 ade8 amp1 cam1 cam2 cam3 cyr1-2*), a *cyr1* mutant defective in adenylate cyclase (19). When grown in the absence of cAMP, this strain has no cAMP-dependent protein kinase activity and is arrested in the G_1 phase of the cell cycle (19). In addition to the defect in adenylate cyclase, strain AM18-5C contains mutations *amp1*, *cam1*, *cam2*, and *cam3* which together facilitate the uptake of cAMP by cells (19). When grown in the presence of 1 mM cAMP, strain AM18-5C grew equally as well as the parent strain wild type for adenylate cyclase, AM3-4B (*MATα ade6 ade8 amp1 cam1 cam2 cam3*). Strain R146-21B (*MATα trp1 ura3 bcy1::URA3*) is a *bcy1* gene disruption mutant defective in the regulatory subunit of cAMP-dependent protein kinase and has high cAMP-dependent protein kinase activity (10, 20). Strain R146-21C (*MATα trp1 ura3 lys2*) is wild type for the regulatory subunit of cAMP-dependent protein kinase.

The levels of cAMP-dependent protein kinase activity can be varied in *cyr1* mutant cells by varying the supplementation of cAMP (19). Mutant *cyr1* (AM18-5C) cells were grown to the mid-exponential phase in 5-ml batches of complete synthetic medium (4, 8) containing 1 mM cAMP. Cells were harvested, washed, and transferred to 5 ml of complete synthetic medium containing the indicated concentrations of cAMP and were incubated for 100 min. Wild-type (AM3-4B) cells were precultured and incubated in medium without cAMP. $^{32}P_i$ (50 μCi) was then added to the cultures, which were incubated for 20 min. All incubations were at 30°C. The labeled cells were harvested by centrifugation and washed with distilled water. Phospholipids were extracted (9) and analyzed by two-dimensional paper chromatography (1, 14). The amount of label in the individual phospholipids was quantitated by scintillation spectroscopy (16). The percentages of $^{32}P_i$ incorporation into each phospholipid represented the relative rates of synthesis during the 20-min pulse (11). Although phosphatidylethanolamine and phosphatidylcholine are major membrane phospholipids (2, 7), they were not heavily labeled during the pulse since they are derived from PS (11). In wild-type cells, the synthesis of phosphatidylinositol (PI) accounted for 44% of the label and the synthesis of PS accounted for 33% of the label (Fig. 1). In contrast, when mutant *cyr1* cells were incubated in the absence of cAMP, the percentage of $^{32}P_i$ found in PS increased to 47% whereas the amount of label found in PI decreased to 32% (Fig. 1). The addition of increasing concentrations of cAMP to the incubation medium of *cyr1* cells resulted in an increase in the rate of PI synthesis at the expense of PS synthesis (Fig. 1). The rates of PI and PS syntheses in *cyr1* cells supplemented with 1 mM cAMP were essentially identical to those in wild-type cells incubated in the absence of cAMP (Fig. 1). The percentages of label recovered in phosphatidylethanolamine, phosphatidylcholine, and minor phospholipids were not significantly affected (Fig. 1).

The effect of cAMP on several phospholipid biosynthesis enzyme activities was examined in mutant *cyr1* (AM18-5C) cells. Cells were incubated in 100-ml batches of medium under conditions identical to those described for the labeling studies. Following incubation, cell extracts were prepared (14) and the activities of PI synthase (3) PS synthase (3) phosphatidylethanolamine methyltransferase (P. M. Gaynor, Ph.D. thesis, Rutgers University, New Brunswick, N.J., 1989), phospholipid methyltransferase (Gaynor, Ph.D. thesis), and CDP-diacylglycerol synthase (12) were measured as previously described. The specific activity of PS

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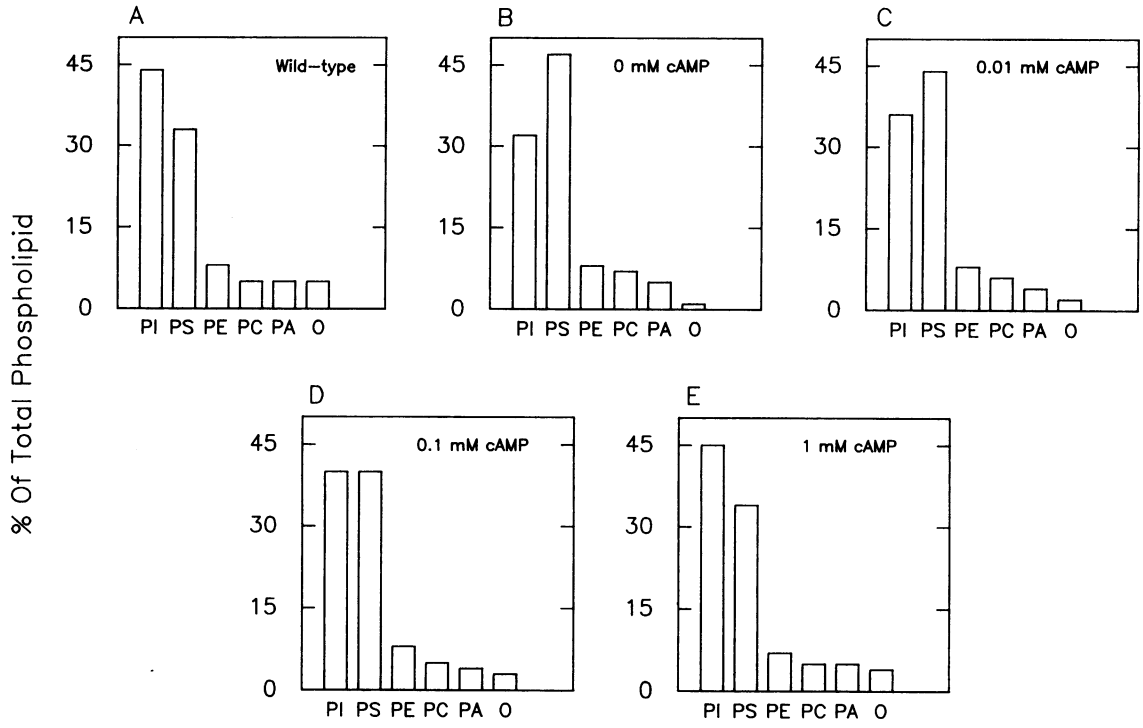

FIG. 1. Pulse-labeling of phospholipids of wild-type and *cyr1* mutant cells. (A) Wild-type (AM3-4B) cells were pulse-labeled with $^{32}P_i$, and the phospholipid composition was analyzed as described in the text. (B through E) Mutant *cyr1* (AM18-5C) cells were pulse-labeled with $^{32}P_i$ in the presence of the indicated concentrations of cAMP, and the phospholipid composition was analyzed as described in the text. The data presented are the averages from at least two independent experiments. PE, Phosphatidylethanolamine; PC, phosphatidylcholine; PA, phosphatidate; O, other (includes the pooled percentages of minor phospholipids).

synthase from mutant *cyr1* cells incubated in the absence of cAMP was 2.6-fold greater than the specific activity of the enzyme from wild-type cells (Fig. 2). Increasing the concentration of cAMP supplementation to *cyr1* mutant cells resulted in a decrease in the specific activity of PS synthase (Fig. 2). PS synthase activity in the *cyr1* mutant supplemented with 1 mM cAMP was the same as that in wild-type cells (Fig. 2). Although the rates of PI synthesis were affected in mutant *cyr1* cells supplemented with cAMP (Fig. 1), PI synthase activity was not significantly affected in these cells (Fig. 2). The results of in vitro and in vivo phosphorylation studies performed as described previously (13), using pure PI synthase (5) and anti-PI synthase antibodies (6), confirmed that PI synthase was not regulated by cAMP-dependent protein kinase phosphorylation. The activities of phosphatidylethanolamine methyltransferase and phospholipid methyltransferase (which are responsible for the synthesis of phosphatidylcholine from phosphatidylethanolamine) and of CDP-diacylglycerol synthase were also not affected in mutant *cyr1* cells supplemented with cAMP (Fig. 2).

The kinetic properties of the phosphorylated form of PS synthase from mutant *bcy1* (R146-21B) cells were examined to characterize the inhibition of activity by cAMP-dependent protein kinase. The apparent V_{max} values for PS synthase with respect to CDP-diacylglycerol (Fig. 3A) and serine (Fig. 3B) from the *bcy1* mutant were about threefold lower than the apparent V_{max} values for the enzyme from wild-type cells. The apparent K_m value of CDP-diacylglycerol (25 μM) for PS synthase from the *bcy1* mutant was twofold lower than the apparent K_m of CDP-diacylglycerol (50 μM) for the enzyme from wild-type cells (Fig. 3A). The apparent K_m value of serine (0.66 mM) for PS synthase from the *bcy1* mutant was the same as the K_m of serine for the enzyme from wild-type cells (Fig. 3B). The specificity constants (V_{max}/K_m) for PS synthase from the *bcy1* mutant were 2.9- and 1.5-fold lower than those for the enzyme from wild-type cells with respect to serine and CDP-diacylglycerol, respectively.

In the yeast *S. cerevisiae*, cell proliferation occurs in the presence of a sufficient nutrient supply. This response is mediated by cAMP (18), which in turn activates cAMP-dependent protein kinase (15). Since cell growth involves a rapid increase in membrane biosynthesis, the effect of cAMP on phospholipid biosynthesis was investigated. We have shown that cAMP and thus cAMP-dependent protein kinase play a role in the regulation of phospholipid biosynthesis in *S. cerevisiae*. The negative effect of cAMP on PS synthesis in vivo correlated with the decrease in PS synthase activity in vitro, which is mediated by cAMP-dependent protein kinase phosphorylation (13). The kinetic experiments showed that the phosphorylation of PS synthase resulted in a decrease in its specificity constants with respect to both substrates. cAMP had a positive effect on PI synthesis in vivo. However, this effect was not due to the regulation of PI synthase activity by cAMP-dependent protein kinase. Therefore, it is likely that the down regulation of PS synthase activity brought about by cAMP-dependent protein kinase phosphorylation resulted in an increase in PI synthesis due to a loss of competition of PI synthase and PS synthase for their common substrate CDP-diacylglycerol. As with the regulation of the partitioning of CDP-diacylglycerol to PI and PS by the water-soluble phospholipid precursor inositol (1, 11, 14, 21), the regulation of phospholipid bio-

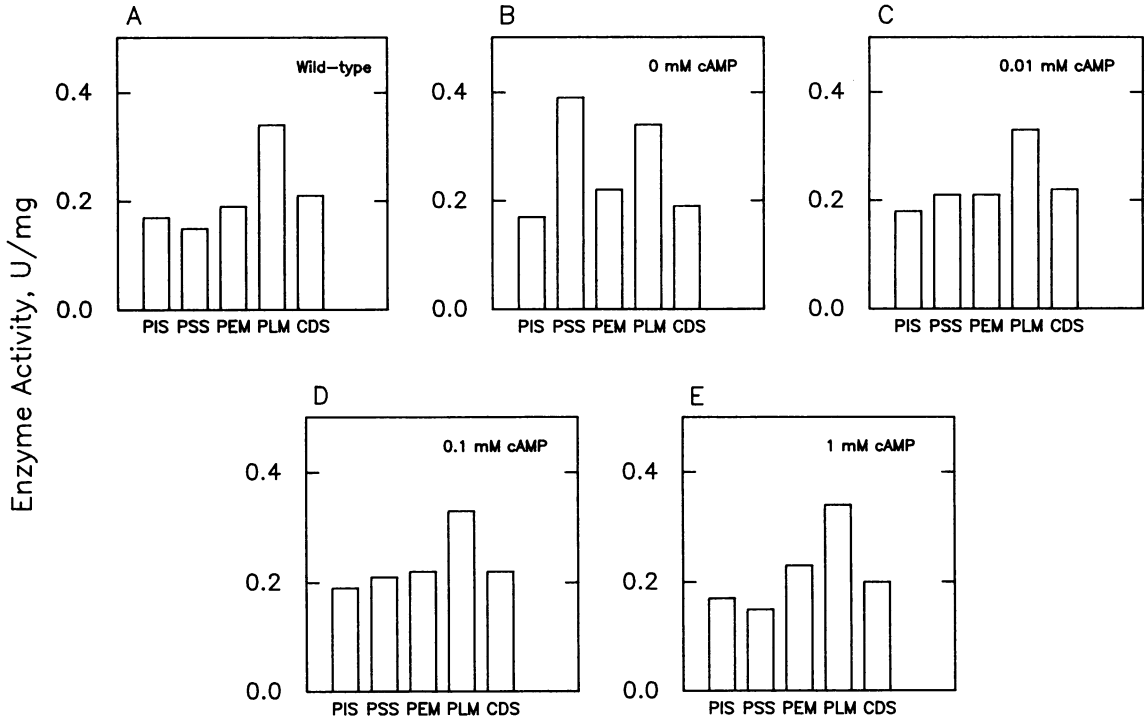

FIG. 2. Phospholipid biosynthesis enzyme activities of wild-type and *cyr1* mutant cells. (A) Wild-type (AM3-4B) cells were incubated as described in the text. (B through E) Mutant *cyr1* (AM18-5C) cells were incubated in the presence of the indicated concentrations of cAMP as described in the text. Cell extracts were prepared and assayed for PI synthase (PIS), PS synthase (PSS), phosphatidylethanolamine methyltransferase (PEM) (units per milligram times 10^{-1}), phospholipid methyltransferase (PLM) (units per milligram times 10^{-1}), and CDP-diacylglycerol synthase (CDS) activities. One unit of enzymatic activity was defined as the amount of enzyme that catalyzed the formation of 1 nmol of product per min. The specific activity was defined as units per milligram of protein.

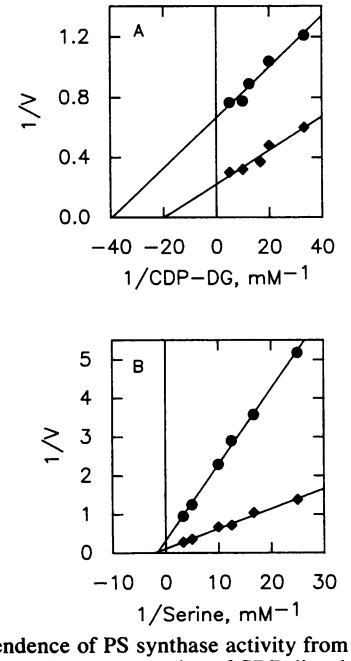

FIG. 3. Dependence of PS synthase activity from wild-type and *bcy1* mutant cells on the concentration of CDP-diacylglycerol (CDP-DG) and serine. Wild-type (R146-21C) (◆) and mutant *bcy1* (R146-21B) (●) cells were grown in complete synthetic medium, and cell extracts were prepared and assayed for PS synthase activity as described in the text. Data are plotted as 1/V (units per milliliter) versus the reciprocal of the CDP-diacylglycerol concentration (A) or the serine concentration (B). The molar ratio of Triton X-100 to CDP-diacylglycerol was maintained at 16:1. The curves drawn were from a least-squares analysis of the data.

synthesis by cAMP-dependent protein kinase favored the synthesis of PI.

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