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Regulation of CDP-Diacylglycerol Synthase Activity in *Saccharomyces cerevisiae*†

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The addition of ethanolamine or choline to inositol-containing growth medium resulted in a reduction of CTP:phosphatidate cytidylyltransferase (CDP-diacylglycerol synthase; EC 2.7.7.41) activity in *Saccharomyces cerevisiae*. The reduction of activity did not occur in the absence of inositol. CDP-diacylglycerol synthase activity was not regulated in a *S. cerevisiae* mutant strain (*opi1*; an inositol biosynthesis regulatory mutant) by the addition of phospholipid precursors to the growth medium.

The liponucleotide CDP-diacylglycerol (CDP-DG) is an important intermediate in the biosynthesis of the major phospholipids in *Saccharomyces cerevisiae* (13). The enzyme responsible for the synthesis of CDP-DG is CTP:phosphatidate cytidylyltransferase (CDP-DG synthase, EC.2.7.7.41) (6). The regulation of this enzyme may be involved in controlling the ionic nature and function of yeast membranes (1). Information on the regulation of phospholipid biosynthesis has come from growth studies of *S. cerevisiae* (10). Some phospholipid biosynthetic enzyme levels are regulated by the addition of water-soluble phospholipid precursors to the growth medium. The presence of the CDP-ethanolamine- and the CDP-choline-based pathways (11) for the synthesis of phosphatidylethanolamine and phosphatidylcholine, respectively (7) prompted us to examine what effects the precursors ethanolamine and choline have on CDP-DG synthase activity in *S. cerevisiae*.

(8, 12). Cells were grown at 28°C in 100 ml of complete synthetic medium (12) containing *myo*-inositol (50 μM), ethanolamine (1 mM), and choline (1 mM) where indicated. Cells were grown to a late-exponential-phase equivalent density of 5 to 10 mg (wet cell weight) per ml. Cells were harvested and cell extracts were prepared as previously described (12).

CDP-DG synthase activity was measured at 30°C as described by Carman et al. (3) by following the incorporation of 1.0 mM [5-^{3}H]CTP (10,000 cpm/nmol) into chloroform-soluble product in the presence of 50 mM MES (morpholineethanesulfonic acid) hydrochloride buffer (pH 6.5)–20 mM $MgCl_2$–0.5 mM phosphatidic acid–5 mM Triton X-100–enzyme protein in a total volume of 0.1 ml. The phospholipid product of the reaction was identified with standard CDP-DG by thin-layer chromatography (3).

CDP-DG synthase activity was measured from the cell extract of the *ade5* and *opi1* strains grown in complete synthetic medium in the absence or presence of water-soluble phospholipid precursors (Table 1). The addition of inositol to the growth medium for the *ade5* strain resulted in a 34% reduction of activity. The addition of both inositol and ethanolamine and both inositol and choline to the growth medium of the *ade5* strain reduced CDP-DG synthase activity 60 and 56%, respectively. Ethanolamine and choline did

TABLE 1. CDP-DG synthase activity in cell extracts from *S. cerevisiae* grown in the presence of phospholipid precursors[a]

Growth condition	Wild type (*ade5*) activity		Mutant (*opi1*) activity	
	U/mg ± SD	Relative activity (%)	U/mg ± SD	Relative activity (%)
Complete synthetic medium (CSM)	0.45 ± 0.02	100	0.78 ± 0.04	100
CSM + inositol	0.30 ± 0.03	66	0.79 ± 0.03	101
CSM + ethanolamine	0.47 ± 0.01	104	0.83 ± 0.02	106
CSM + inositol + ethanolamine	0.18 ± 0.04	40	0.82 ± 0.07	105
CSM + choline	0.45 ± 0.03	100	0.75 ± 0.10	96
CSM + inositol + choline	0.20 ± 0.01	44	0.81 ± 0.06	104

[a] *S. cerevisiae ade5* and *opi1* strains were grown in complete synthetic medium with 50 μM inositol, 1 mM ethanolamine, and 1 mM choline where indicated. The specific activities (units per milligram) of CDP-DG synthase were calculated from triplicate determinations from a minimum of two independent growth studies. One unit of enzymatic activity was defined as the amount of enzyme that catalyzed the formation of 1 nmol of product per min under the assay conditions described in the text. Protein was determined by the method of Bradford (2) with bovine serum albumin as the standard.

The mutant *S. cerevisiae opi1-1 ade5 MAT***a** strain is constitutive for inositol biosynthesis (8) and other enzymes of phospholipid biosynthesis (12). The parent *ade5 MAT***a** strain shows normal regulation of phospholipid biosynthesis

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not inhibit activity when added to medium without inositol. The *opi1* phospholipid biosynthetic regulatory mutant (8, 9, 12) was completely constitutive for CDP-DG synthase activity and failed to respond to inositol alone or in combination with ethanolamine and choline (Table 1). In addition, the specific activity of CDP-DG synthase in the *opi1* mutant was elevated about 1.7-fold above the wild-type *ade5* level.

A similar pattern of regulation has been shown for other enzymes of phospholipid biosynthesis in *S. cerevisiae*. For example, the membrane-associated phospholipid *N*-methyltransferases which convert phosphatidylethanolamine to phosphatidylcholine are repressed 6- to 10-fold when choline is added to medium already containing inositol (14). However, the growth of cells in the presence of choline in medium lacking inositol results in no repression of these enzymes (15). Similarly, both phosphatidylserine synthase (4) and phosphatidylserine decarboxylase (5) are repressed about three- to fourfold by the addition of choline to medium containing inositol. However, it has recently been demonstrated that phosphatidylserine synthase, like the phospholipid *N*-methyltransferases, is repressed by choline only in the presence of inositol (12). Thus, in *S. cerevisiae* a number of enzymatic activities involved in phospholipid synthesis have been shown to be regulated in a coordinate fashion in response to inositol and choline.

The *opi1* mutant, which was originally described as constitutive for inositol-1-phosphate synthase (8, 9), was found to be pleiotropically constitutive for phosphatidylserine synthase and the phospholipid *N*-methyltransferases (12). Consistent with these observations, in the present study CDP-DG synthase activity was found to be constitutive in the *S. cerevisiae opi1* mutant strain.

The results of these studies suggest that the regulatory mechanism governing the control of inositol biosynthesis pleiotropically regulates CDP-DG synthase activity in *S. cerevisiae*. The fact that CDP-DG synthase activity was slightly elevated in the *opi1* mutant and was expressed at the same level whether or not water-soluble phospholipid precursors were present supports the idea that the *OPI1* gene product is a negative regulator (8, 9, 12). As a consequence of the reduction in *S. cerevisiae* CDP-DG synthase activity by ethanolamine or choline in inositol-containing medium, the CDP-ethanolamine- or CDP-choline-based pathways (11) may be activated to synthesize required levels of phosphatidylethanolamine or phosphatidylcholine, respectively.

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LITERATURE CITED

1. **Becker, G. W., and R. L. Lester.** 1977. Changes in phospholipids of *Saccharomyces cerevisiae* associated with inositol-less death. J. Biol. Chem. **252**:8684–8691.
2. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
3. **Carman, G. M., R. L. Zaniewski, and J. J. Cousminer.** 1982. CDP-diacylglycerol synthase activity in *Clostridium perfringens*. Appl. Environ. Microbiol. **43**:81–85.
4. **Carson, M. A., K. D. Atkinson, and C. J. Waechter.** 1982. Properties of particulate and solubilized phosphatidylserine synthase activity from *Saccharomyces cerevisiae*. Inhibitory effect of choline in the growth medium. J. Biol. Chem. **257**:8115–8121.
5. **Carson, M. A., M. Emala, P. Hogsten, and C. J. Waechter.** 1984. Coordinate regulation of phosphatidylserine decarboxylase activity and phospholipid *N*-methylation in yeast. J. Biol. Chem. **259**:6267–6273.
6. **Carter, J. R., Jr.** 1968. Cytidine triphosphate:phosphatidic acid cytidylyltransferase in *Escherichia coli*. J. Lipid Res. **9**:748–754.
7. **Christiansen, K.** 1979. Utilization of endogenous diacylglycerol for the synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine by lipid particles from baker's yeast (*Saccharomyces cerevisiae*). Biochim. Biophys. Acta **574**: 448–460.
8. **Greenberg, M., P. Goldwasser, and S. A. Henry.** 1982. Characterization of a yeast regulatory mutant constitutive for inositol-1-phosphate synthase. Mol. Gen. Genet. **186**:157–163.
9. **Greenberg, M., B. Reiner, and S. A. Henry.** 1982. Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol excreting mutants. Genetics **100**:19–33.
10. **Henry, S. A., L. S. Klig, and B. S. Loewy.** 1984. Genetic regulation and coordination of biosynthetic pathways in yeast: amino acid and phospholipid synthesis. Annu. Rev. Genet. **18**:207–231.
11. **Kennedy, E. P., and S. B. Weiss.** 1956. The function of cytidine coenzymes in the biosynthesis of phospholipids. J. Biol. Chem. **222**:193–214.
12. **Klig, L. S., M. J. Homann, G. M. Carman, and S. A. Henry.** 1985. Coordinate regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*: pleiotropically constitutive *opi1* mutant. J. Bacteriol. **162**:1135–1141.
13. **Steiner, M. R., and R. L. Lester.** 1972. In vitro studies of phospholipid biosynthesis in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **260**:222–243.
14. **Waechter, C. J., and R. L. Lester.** 1973. Differential regulation of the N-methyl transferases responsible for phosphatidylcholine synthesis in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **158**:401–410.
15. **Yamashita, S., A. Oshima, J. Kawa, and K. Hosaka.** 1982. Regulation of the phosphatidylethanolamine methylation pathway in *Saccharomyces cerevisiae*. Eur. J. Biochem. **128**: 589–595.