JOURNAL OF BACTERIOLOGY, July 1990, p. 4115–4117
0021-9193/90/074115-03$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 172, No. 7

Enzymes of Phosphoinositide Synthesis in Secretory Vesicles Destined for the Plasma Membrane in *Saccharomyces cerevisiae*†

ANTHONY J. KINNEY‡ AND GEORGE M. CARMAN*

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903

Received 20 February 1990/Accepted 26 April 1990

CDP-diacylglycerol synthase, phosphatidylinositol synthase, and phosphatidylinositol kinase activities were associated with post-Golgi apparatus secretory vesicles destined for the plasma membrane of *Saccharomyces cerevisiae*. These results suggest that the plasma membrane is capable of synthesizing both CDP-diacylglycerol and phosphatidylinositol as well as phosphorylating phosphatidylinositol.

The response of eucaryotic cells, including the yeast *Saccharomyces cerevisiae* (27), to external signals is mediated by the turnover of phosphatidylinositol and its phosphorylated derivatives, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate (4). Polyphosphoinositide formation and breakdown are localized in the plasma membranes of animals (4) and *S. cerevisiae* (20, 27). However, the resynthesis of phosphatidylinositol by the enzymes CDP-diacylglycerol synthase and phosphatidylinositol synthase has been thought to be confined to the endoplasmic reticulum, mitochondria, and nuclear membranes (4, 22). It has been suggested that newly synthesized phosphatidylinositol is transported from the endoplasmic reticulum to the plasma membrane by phospholipid transfer proteins (10–12). Recently, however, phosphatidylinositol synthase activity was measured in plasma membranes isolated from GH_3 pituitary cells (18). In the present study, we showed that in *S. cerevisiae*, CDP-diacylglycerol synthase, phosphatidylinositol synthase, and phosphatidylinositol kinase are localized in purified secretory vesicles destined for the plasma membrane.

Our studies were facilitated by the use of strain NY17 (*MAT***a** *ura3-52 sec6-4*), a *sec6-4* temperature-sensitive mutant that accumulates post-Golgi apparatus secretory vesicles at the restrictive temperature (28). These vesicles contain proteins destined for the plasma membrane (6, 28) and are required for membrane growth in *S. cerevisiae* (25). Secretory vesicles can be highly purified from the spheroplasts of temperature-shifted *sec6-4* mutants (28). Unlike plasma membrane preparations from *S. cerevisiae* (5, 26), purified secretory vesicles from *sec6-4* mutant cells are not significantly contaminated with endoplasmic reticulum membranes (28). *sec6-4* mutant strain NY17 and the parent wild-type strain NY13 (*MAT***a** *ura3-52*) were grown to the mid-exponential phase in 1% yeast extract–2% peptone–2% glucose medium at 25°C (28). Cells were transferred to 1% yeast extract–2% peptone–0.2% glucose medium prewarmed to 37°C and were incubated at this temperature for 2 h. Spheroplasts were prepared from the temperature-shifted cells in buffer containing 1.4 M sorbitol and were lysed by transfer to buffer containing 8 M sorbitol (28). Microsomes were prepared from spheroplasts of wild-type and *sec6-4* mutant cells by differential centrifugation (28). Secretory vesicles were purified from microsomes derived from *sec6-4* mutant cells by Sephacryl S-1000 gel filtration chromatography as described by Walworth and Novick (28). The purification of secretory vesicles was monitored by the measurement of invertase activity (16). The contamination of endoplasmic reticulum membranes in the purified secretory vesicles was assessed at less than 1% by using NADPH cytochrome *c* reductase activity (13) as an endoplasmic reticulum marker (28). CDP-diacylglycerol synthase (21), phosphatidylinositol synthase (14), phosphatidylinositol kinase (3), and phosphatidylserine synthase (1) activities were measured as previously described. Protein was determined (7) with bovine serum albumin as the standard. CDP-diacylglycerol synthase (21), phosphatidylinositol synthase (15), and phosphatidylserine synthase (24) subunits were identified by immunoblot analysis with specific antibodies to each enzyme.

The activity of the enzymes involved in phosphoinositide synthesis was measured in microsomes derived from wild-type and *sec6-4* mutant cells (Table 1). As previously described, CDP-diacylglycerol synthase (21), phosphatidylinositol synthase (14), and phosphatidylinositol kinase (3) activities were found in microsomes derived from wild-type cells. The specific activities of each of these enzymes were not significantly different in microsomes derived from temperature-shifted *sec6-4* mutant cells (Table 1). Secretory vesicles were purified from microsomes derived from *sec6-4* mutant cells, and the biosynthetic enzyme activities were measured. CDP-diacylglycerol synthase, phosphatidylinositol synthase, and phosphatidylinositol kinase activities were found in the purified secretory vesicles. The specific activity of CDP-diacylglycerol was 5.5-fold greater in secretory vesicles than in microsomes (Table 1). Phosphatidylinositol synthase and phosphatidylinositol kinase activities were not significantly enriched in vesicles as compared with microsomes derived from *sec6-4* mutant cells (Table 1). Immunoblot analysis confirmed that the CDP-diacylglycerol synthase 56,000- and 54,000-M_r subunits (21) were associated with microsomes and purified vesicles derived from *sec6-4* mutant cells (Fig. 1). There was a qualitative enrichment of the CDP-diacylglycerol synthase subunits in the purified vesicles consistent with the enrichment of CDP-diacylglycerol synthase specific activity. Immunoblot analysis also confirmed the presence of the phosphatidylinositol synthase 34,000-M_r subunit (14) in purified secretory vesicles from

* Corresponding author.

† New Jersey Agricultural Experiment Station publication D-10531-2-90.

‡ Present address: Agricultural Products Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19880-0402.

TABLE 1. Phospholipid biosynthesis enzyme activities in microsomes and purified vesicles[a]

Strain (membrane fraction)	Sp act (nmol/min per mg) of:			
	CDP-DG synthase	PI synthase	PI kinase	PS synthase
Wild type (microsomes)	0.14	0.34	0.73	0.18
sec6-4 mutant (microsomes)	0.12	0.32	0.75	0.13
sec6-4 mutant (vesicles)	0.66	0.43	0.77	<0.01

[a] Microsomes and vesicles were prepared from wild-type (NY13) and *sec6-4* mutant (NY17) cells as described in the text. CDP-diacylglycerol (CDP-DG) synthase, phosphatidylinositol (PI) synthase, PI kinase, and phosphatidylserine (PS) synthase activities are the averages of three determinations.

sec6-4 mutant cells (data not shown). The level of the phosphatidylinositol synthase subunit in the vesicles was not significantly different from that in the microsomes.

Phosphatidylserine synthase activity is associated with the mitochondria and microsome fractions of *S. cerevisiae* (22). This enzyme is not involved in phosphoinositide biosynthesis (8) and was used as a control. Phosphatidylserine synthase activity was found in microsomes derived from wild-type and *sec6-4* mutant cells but was not found in secretory vesicles derived from *sec6-4* microsomes (Table 1). In addition, the phosphatidylserine synthase subunit was not detected by immunoblot analysis in purified secretory vesicles (data not shown). These results along with the NADPH cytochrome *c* reductase activity measurements confirmed that our secretory vesicle preparation was not significantly contaminated with endoplasmic reticulum membranes.

Secretory vesicles are required for plasma membrane assembly (6, 25) and contain plasma membrane-associated proteins such as ATPase (6). The vesicles do not contain endoplasmic reticulum-associated proteins such as NADPH cytochrome *c* reductase (28). Therefore, the association of CDP-diacylglycerol synthase and phosphatidylinositol synthase with the secretory vesicles supports the notion that the plasma membrane in *S. cerevisiae* is capable of synthesizing both CDP-diacylglycerol and phosphatidylinositol as well as phosphorylating phosphatidylinositol. The main site of CDP-diacylglycerol synthesis in *S. cerevisiae* is the mitochondria (21, 22). About 20% of CDP-diacylglycerol synthase activity is associated with microsomes (21). The activity of this enzyme, unlike that of phosphatidylinositol synthase and phosphatidylinositol kinase, was enriched 5.5-fold in the secretory vesicles as compared with the microsomes of *sec6-4* mutant cells. This observation suggests that the CDP-diacylglycerol synthase activity in microsomes is contributed mainly by plasma membrane-associated activity.

Phosphatidylinositol is rapidly phosphorylated and hydrolyzed during a signal response in *S. cerevisiae* (9, 19, 27) and in higher eucaryotes (4). The rapid synthesis of phosphatidylinositol may be necessary to maintain a steady-state concentration of this essential membrane phospholipid in *S. cerevisiae* (2, 17, 23). This study supports the notion that phosphatidylinositol synthesis via CDP-diacylglycerol synthase and phosphatidylinositol synthase may occur in the plasma membrane. This study does not rule out the necessity of participation of intracellular organelles or the requirement of a phosphatidylinositol transfer protein (10–12) for plasma membrane-associated phosphoinositide synthesis in *S. cerevisiae*.

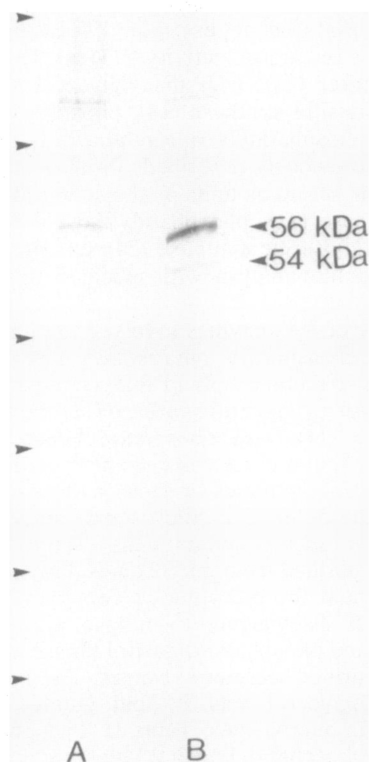

FIG. 1. Immunoblot analysis of CDP-diacylglycerol synthase subunits from microsomes and vesicles isolated from temperature-shifted *sec6-4* mutant cells. Microsomes and vesicles were isolated from temperature-shifted *sec6-4* mutant cells as described in the text. Immunoblotting of 30 μg each of microsomes (lane A) and vesicles (lane B) was performed with antibodies specific for CDP-diacylglycerol synthase as described in the text. Protein molecular mass standards (indicated by the arrowheads) were phosphorylase *b* (92.5 kilodaltons [kDa]), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor 21.5 kDa), and lysozyme (14.4 kDa).

This work was supported by Public Health Service grant GM-28140 from the National Institutes of Health, New Jersey State funds, and the Charles and Johanna Busch Memorial Fund.

We thank Peter Novick for providing us with yeast strains NY13 and NY17.

LITERATURE CITED

1. **Bae-Lee, M., and G. M. Carman.** 1984. Phosphatidylserine synthesis in *Saccharomyces cerevisiae*. Purification and characterization of membrane-associated phosphatidylserine synthase. J. Biol. Chem. **259**:10857–10862.
2. **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.
3. **Belunis, C. J., M. Bae-Lee, M. J. Kelley, and G. M. Carman.** 1988. Purification and characterization of phosphatidylinositol kinase from *Saccharomyces cerevisiae*. J. Biol. Chem. **263**: 18897–18903.
4. **Berridge, M. J.** 1987. Inositol trisphosphate and diacylglycerol: two interacting second messengers. Annu. Rev. Biochem. **56**: 159–193.
5. **Bottema, C. D. K., C. A. McLean-Bowen, and L. W. Parks.** 1983. Role of sterol structure in the thermotropic behavior of

plasma membranes of *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **734**:235–248.

6. **Brada, D., and R. Schekman.** 1988. Coincident localization of secretory and plasma membrane proteins in organelles of the yeast secretory pathway. J. Bacteriol. **170**:2775–2783.
7. **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.
8. **Carman, G. M., and S. A. Henry.** 1989. Phospholipid biosynthesis in yeast. Annu. Rev. Biochem. **58**:635–669.
9. **Dahl, J. S., and C. E. Dahl.** 1985. Stimulation of cell proliferation and polyphosphoinositide metabolism in *Saccharomyces cerevisiae* GL7 by ergosterol. Biochem. Biophys. Res. Commun. **133**:844–850.
10. **Daum, G., E. Heidorn, and F. Paltauf.** 1986. Intracellular transfer of phospholipids in the yeast, *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **878**:93–101.
11. **Daum, G., and F. Paltauf.** 1984. Phospholipid transfer in yeast. Isolation and partial characterization of a phospholipid transfer protein from yeast cytosol. Biochim. Biophys. Acta **794**:385–391.
12. **Daum, G., H. G. Schwelberger, and F. Paltauf.** 1986. Stability and effects of culture conditions on the cellular content and the activity of a phosphatidylcholine/phosphatidylinositol transfer protein from *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **879**:240–246.
13. **Dreibich, G., P. Debey, and D. D. Sabatini.** 1973. Selective release of content from microsomal vesicles without membrane disassembly. I. Permeability changes induced by low detergent concentrations. J. Cell Biol. **58**:436–462.
14. **Fischl, A. S., and G. M. Carman.** 1983. Phosphatidylinositol biosynthesis in *Saccharomyces cerevisiae*: purification and properties of microsome-associated phosphatidylinositol synthase. J. Bacteriol. **154**:304–311.
15. **Fischl, A. S., M. J. Homann, M. A. Poole, and G. M. Carman.** 1986. Phosphatidylinositol synthase from *Saccharomyces cerevisiae*. Reconstitution, characterization, and regulation of activity. J. Biol. Chem. **261**:3178–3183.
16. **Goldstein, A., and J. O. Lampen.** 1975. β-D-Fructofuranoside fructohydrolase from yeast. Methods Enzymol. **42**:504–577.
17. **Henry, S. A., K. D. Atkinson, A. J. Kolat, and M. R. Culbertson.** 1977. Growth and metabolism of inositol-starved *Saccharomyces cerevisiae*. J. Bacteriol. **130**:472–484.
18. **Imai, A., and M. C. Gershengorn.** 1987. Independent phosphatidylinositol synthesis in pituitary plasma membrane and endoplasmic reticulum. Nature (London) **325**:726–728.
19. **Kaibuchi, K., A. Miyajima, K.-I. Arai, and K. Matsumoto.** 1986. Possible involvement of RAS-encoded proteins in glucose-induced inositolphospholipid turnover in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **83**:8172–8176.
20. **Kato, H., I. Uno, T. Ishikawa, and T. Takenawa.** 1989. Activation of phosphatidylinositol kinase and phosphatidylinositol-4-phosphate kinase by cAMP in *Saccharomyces cerevisiae*. J. Biol. Chem. **264**:3116–3121.
21. **Kelley, M. J., and G. M. Carman.** 1987. Purification and characterization of CDPdiacylglycerol synthase from *Saccharomyces cerevisiae*. J. Biol. Chem. **262**:14563–14570.
22. **Kuchler, K., G. Daum, and F. Paltauf.** 1986. Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. J. Bacteriol. **165**:901–910.
23. **Nikawa, J., T. Kodaki, and S. Yamashita.** 1987. Primary structure and disruption of the phosphatidylinositol synthase gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **262**:4876–4881.
24. **Poole, M. A., M. J. Homann, M. Bae-Lee, and G. M. Carman.** 1986. Regulation of phosphatidylserine synthase from *Saccharomyces cerevisiae* by phospholipid precursors. J. Bacteriol. **168**:668–672.
25. **Ramirez, R. M., T. Ishida-Schick, B. L. Krilowicz, B. A. Leish, and K. D. Atkinson.** 1983. Plasma membrane expansion terminates in *Saccharomyces cerevisiae* secretion-defective mutants while phospholipid synthesis continues. J. Bacteriol. **154**:1276–1283.
26. **Schmidt, R., R. Ackermanm, Z. Kratky, B. Wasserman, and B. Jacobson.** 1983. Fast and efficient purification of yeast plasma membranes using cationic silica microbeads. Biochim. Biophys. Acta **732**:421–427.
27. **Uno, I., K. Fukami, H. Kato, T. Takenawa, and T. Ishikawa.** 1988. Essential role for phosphatidylinositol 4,5-bisphosphate in yeast cell proliferation. Nature (London) **333**:188–190.
28. **Walworth, N. C., and P. J. Novick.** 1987. Purification and characterization of constitutive secretory vesicles from yeast. J. Cell Biol. **105**:163–174.