Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 23, Issue of June 7, pp. 13293–13296, 1996

Regulation of Phospholipid Biosynthesis in the Yeast *Saccharomyces cerevisiae**

George M. Carman‡ and Geri Marie Zeimetz

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

Phospholipids are key molecules that contribute to the structural definition of cells and that participate in the regulation of cellular processes. Phospholipid metabolism is a major activity that cells engage in throughout their growth. The yeast, *Saccharomyces cerevisiae*, serves as a model system in which to study the regulation of phospholipid synthesis and its regulation in eucaryotes. Its membranous organelles, the lipids that comprise these membranes, and the phospholipid biosynthetic pathways that generate these membranes typify eucaryotic cells (1, 2). Many of the structural genes encoding for the phospholipid biosynthetic enzymes have been cloned and characterized (Table I) (3–25), and a number of mutations in these genes have been isolated (3, 7–9, 11–13, 17, 23, 26–33). In addition, a number of the phospholipid biosynthetic enzymes have been purified and studied (Table I) (34–44). The characterization of the wild-type and mutant genes, as well as the gene products encoded by these alleles, has significantly advanced our understanding both of phospholipid biosynthesis and of its regulation. Results from these genetic, molecular, and biochemical studies have shown that the regulation of phospholipid synthesis is a complex, highly coordinated process. The mechanisms that govern this regulation mediate the mRNA and protein levels of the biosynthetic enzymes as well as their activity and localization (1, 2, 45). This review summarizes our current understanding of the regulation of phospholipid metabolism in *S. cerevisiae* with a particular focus on the regulation of the activity of the biosynthetic enzymes. For more comprehensive reviews, the reader is directed to recent articles by Paltauf *et al.* (2) and Greenberg and Lopes (45).

Phospholipid Composition of S. cerevisiae

The major phospholipids found in mitotically growing cells are PC,[1] PE, PI, and PS (2). Phospholipid composition can vary dramatically when culture conditions are altered (2). Examples of this include: inositol supplementation of wild-type cells (46, 47); inositol starvation of *ino1* mutant cells (48–50); choline/ethanolamine starvation of *cho1* mutant cells (51); fumonisin B_1 supplementation of wild-type cells (52); and glucose starvation of wild-type and respiratory deficient cells (53, 54). Although the proportions of the individual phospholipids change with these growth conditions, the average charge of the membrane phospholipids remains relatively constant (2, 48). Therefore, mechanisms exist in *S. cerevisiae* that compensate for changes in the levels of phospholipids of one charge by orchestrating parallel changes in the levels of phospholipids of the opposite charge. The mechanisms that mediate these processes and other aspects of phospholipid metabolism include genetic regulation and biochemical regulation of the phospholipid biosynthetic enzymes.

Phospholipid Biosynthetic Pathways

Phospholipid biosynthesis is a complex process that contains a number of branch points (Fig. 1). PS, PE, and PC are synthesized from PA by the CDP-DG pathway (indicated in Fig. 1 by the color *blue*), while PE and PC are also synthesized by the Kennedy (CDP-choline and CDP-ethanolamine) pathway (indicated in Fig. 1 by the color *red*) (1, 2, 55, 56). CDP-DG is also used for the synthesis of other phospholipids, including inositol-containing lipids (phosphoinositides and sphingolipids) and CL. The CDP-DG pathway is used by wild-type cells for the synthesis of PE and PC when they are grown in the absence of ethanolamine or choline (1, 2, 57, 58). The Kennedy pathway assumes a critical role in PC synthesis when the enzymes in the CDP-DG pathway are defective or repressed (1, 2, 45). Mutants defective in the CDP-DG pathway require choline for growth and synthesize PC via CDP-choline (9–12, 26, 29, 59, 60). Mutants defective in PS synthase (26, 59) and PS decarboxylase (9, 60) can also synthesize PC if they are supplemented with ethanolamine. Under these conditions, PE is synthesized from CDP-ethanolamine. The PE may be subsequently methylated by the phospholipid *N*-methyltransferases to form PC (Fig. 1). It is not clear what the relative contributions of the CDP-DG and Kennedy pathways are to PE and PC synthesis when ethanolamine and/or choline is present in the growth media.

The utilization of the CDP-DG and Kennedy pathways is also regulated by the cellular levels of CTP (61). The elevation of cellular levels of CTP results in a 2-fold increase in the utilization of the Kennedy pathway for PC synthesis. This has been attributed to an increase in substrate availability for the choline-P cytidylyltransferase reaction in the Kennedy pathway and the inhibition of PS synthase activity by CTP in the CDP-DG pathway (61).

Regulation of Phospholipid Biosynthesis

A number of factors regulate phospholipid biosynthesis including inositol, choline, ethanolamine, lipids (*e.g.* PA and CDP-DG), nucleotides (*e.g.* ATP and CTP), and growth phase. The regulation of phospholipid biosynthetic enzymes by inositol has been the most extensively characterized (2, 45).

Inositol Effects on the CDP-DG and Kennedy Pathways—The addition of inositol to the growth medium of wild-type cells alters phospholipid composition. The level of PI increases while the levels of PS, PE, and PC decrease (46, 47). These changes are due in part to repression mechanisms. These mechanisms regulate mRNA and protein levels and/or the activity of the phospholipid biosynthetic enzymes. For example, the activity and/or levels of the CDP-DG pathway enzymes (*i.e.* CDP-DG synthase (62, 63), PS synthase (46, 64–66), PS decarboxylase (67–69), and the two phospholipid *N*-methyltransferases (46, 67, 70–73)) are reduced when wild-type cells are supplemented with inositol. In many instances, the repressive effects of inositol are enhanced by the inclusion of ethanolamine or choline in the growth medium. This regulation is absolutely dependent on inositol (1, 2, 45). Under these growth conditions, the exogenous ethanolamine and choline is used to synthesize PE and PC via the Kennedy pathway (1, 2). The coordinate regulation of the CDP-DG pathway enzymes by inositol requires ongoing PC synthesis (70, 74). Data from recent studies have shown that, even in the absence of exogenous ethanolamine and choline, the Kennedy pathway contributes to the synthesis of PC (61, 75, 76). Data suggest that the choline required is derived from the turnover of PC synthesized by the CDP-DG pathway (75, 76). This may indicate that the PC generated by each pathway has distinct as well as overlapping functions in cell physiology. The relative contributions of the Kennedy and CDP-DG pathways to phospholipid synthesis in the absence of exogenous ethanolamine or choline are not known. An apparent paradox in the regulation of

* This minireview will be reprinted in the 1996 Minireview Compendium, which will be available in December, 1996. This work was supported by United States Public Health Service Grants GM-28140, GM-35655, and GM-50679 from the National Institutes of Health, New Jersey State funds, and the Charles and Johanna Busch Memorial Fund.
‡ To whom correspondence and reprint requests should be addressed. Tel.: 908-932-9611 (ext. 217); Fax: 908-932-6776; E-mail: carman@aesop.rutgers.edu.
[1] The abbreviations used are: PC, phosphatidylcholine; PA, phosphatidate; PS, phosphatidylserine; PE, phosphatidylethanolamine; DG, diacylglycerol; TG, triacylglycerol; PI, phosphatidylinositol; PIP, PI 4-phosphate; PIP_2, PI 4,5-bisphosphate; IPC, inositol phosphorylceramide; MIPC, mannosylinositol phosphorylceramide; $M(IP)_2C$, mannosyldiinositol phosphorylceramide; PGP, phosphatidylglycerophosphate; CL, cardiolipin; DGPP, diacylglycerol pyrophosphate.

TABLE I

Phospholipid biosynthetic genes cloned and enzymes purified

The table lists the phospholipid biosynthetic genes cloned and enzymes purified to near homogeneity.

Gene	Enzyme	Cloned[a]	Purified[a]
CDS1	CDP-DG synthase	3	34
CHO1/PSS	PS synthase	4–6	35
PSD1	PS decarboxylase 1	7, 8	NP
PSD2	PS decarboxylase 2	9	NP
PEM1/CHO2	PE methyltransferase	10, 11	NP
PEM2/OPI3	Phospholipid methyltransferase	10, 12	NP
	45-kDa PA phosphatase	NC	37
	104-kDa PA phosphatase	NC	36, 37
EPT1	Ethanolamine-phosphotransferase	13, 14	NP
CKI	Choline kinase	15	NP
CCT	Choline-P cytidylyltransferase	16	NP
CPT1	Cholinephospho-transferase	17, 18	NP
INO1	Inositol-1-P synthase	19, 20	38
PIS	PI synthase	21, 22	39
	45-kDa PI 4-kinase	NC	40, 41
	55-kDa PI 4-kinase	NC	42
PIK1	125-kDa PI 4-kinase	23	43
VPS34	PI 3-kinase	24, 25	NP
	DGPP phosphatase	NC	44

[a] Numbers are reference numbers. NP, not purified; NC, not cloned.

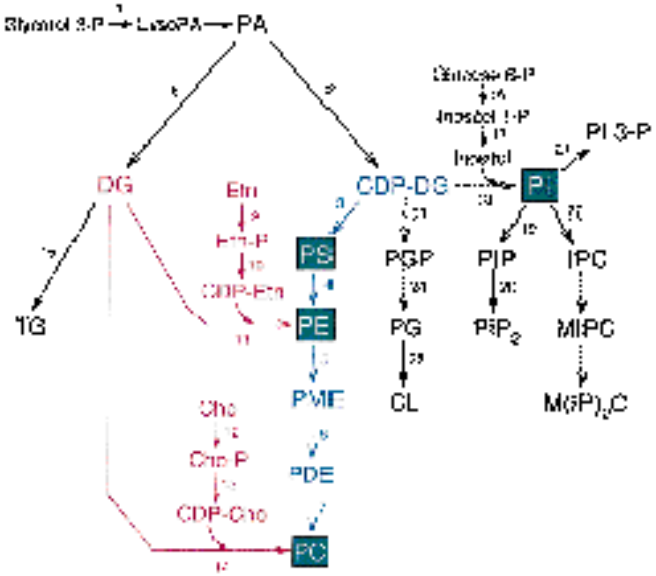

FIG. 1. **Phospholipid biosynthetic pathways in *S. cerevisiae*.** The indicated reactions are catalyzed by the following enzymes: *1*, glycerol-3-P acyltransferase; *2*, CDP-DG synthase; *3*, PS synthase; *4*, PS decarboxylase; *5*, PE methyltransferase; *6* and *7*, phospholipid methyltransferase; *8*, PA phosphatase; *9*, ethanolamine kinase; *10*, ethanolamine-P cytidylyltransferase; *11*, ethanolaminephosphotransferase; *12*, choline kinase; *13*, choline-P cytidylyltransferase; *14*, cholinephosphotransferase; *15*, DG acyltransferase; *16*, inositol-1-P synthase; *17*, inositol-1-P phosphatase; *18*, PI synthase; *19*, PI 4-kinase; *20*, PIP kinase; *21*, PI 3-kinase; *22*, IPC synthase; *23*, PGP synthase; *24*, PGP phosphatase; and *25*, CL synthase. The CDP-DG pathway is indicated by the color *blue* and the Kennedy pathway is indicated by the color *red*. *Etn*, ethanolamine; *Cho*, choline; *PME*, phosphatidylmonomethylethanolamine; *PDE*, phosphatidyldimethylethanolamine; *PG*, phosphatidylglycerol. The four major phospholipids (PC, PE, PI, and PS) are indicated by *green boxes*.

phospholipid synthesis is the repression by inositol of the mRNA abundance of the Kennedy pathway enzymes choline kinase (77), cholinephosphotransferase (78), and ethanolaminephosphotransferase (74). In addition, the inositol (79, 80) and choline (81) transporters are repressed by inositol. If the Kennedy pathway is needed for PE and PC synthesis when the CDP-DG pathway enzymes are repressed, then why are these enzymes repressed?

Cross-regulation of the Pathways for the Synthesis of PI and PC—The level of inositol 1-phosphate synthase (encoded by the *INO1* gene) is reduced in cells supplemented with inositol, and this effect is enhanced by the addition of choline (19, 20, 32, 38, 82). Thus, inositol regulates enzymes in the pathways leading to the synthesis of PI and PC suggesting that these pathways are coordinately regulated (1, 2). In fact, data indicate that at least one level of coordinate regulation exists that involves the transcriptional regulators Ino2p, Ino4p, and Opi1p (1, 2, 45). For example, Ino2p and Ino4p activate the expression of the genes encoding for inositol 1-phosphate synthase and PS synthase, while Opi1p represses the expression of these genes (32, 38, 46, 64, 65, 83–89). In contrast, PI synthase, which utilizes inositol for the synthesis of PI, is not regulated by inositol alone or in combination with ethanolamine or choline (46, 63, 90). However, IPC synthase, which utilizes PI for the synthesis of sphingolipids, is regulated by inositol (91). IPC synthase activity is elevated in wild-type cells supplemented with inositol, and this effect is dependent on the *INO4* regulatory gene (91).

Inositol effects are also observed with enzymes that function at earlier steps in the biosynthetic pathway. One example of this is PA phosphatase. PA phosphatase catalyzes the formation of DG. DG is used for the CDP-ethanolamine- and CDP-choline-based reactions in the Kennedy pathway (Fig. 1) (1, 2). Two membrane-associated forms of PA phosphatase (45 and 104 kDa) have been identified in *S. cerevisiae* (36, 37). The addition of inositol to the growth medium of wild-type cells results in the elevation of the levels of the 45-kDa PA phosphatase, while the levels of the 104-kDa PA phosphatase are not altered (37). Choline, in the absence or presence of inositol, has no effect on the PA phosphatases (37). Mutations in genes (*OPI1*, *INO2*) that alter the expression of *INO1* also influence the levels of the 45-kDa PA phosphatase (37, 92). These observations are consistent with a model that predicts that the expression of the gene that encodes this PA phosphatase is regulated in response to inositol.

Biochemical Regulation of Phospholipid Biosynthetic Enzymes

The rapid changes in the rates of phospholipid synthesis in response to inositol supplementation (47), choline/ethanolamine starvation of *cho1* mutant cells (51), fumonisin B_1 supplementation of wild-type cells (52), and glucose starvation of wild-type and respiratory deficient cells (53, 54) cannot be simply ascribed to genetic mechanisms. It is likely that the direct regulation of enzyme activities also mediates phospholipid synthesis. A number of the biosynthetic enzymes (*e.g.* CDP-DG synthase (34), PS synthase (35), PA phosphatase (36, 37), PI synthase (39), and PI 4-kinase) have been purified to near homogeneity, and defined studies of their biochemical regulation have been conducted (Table II). This regulation will be discussed in the context of phospholipid synthesis.

Regulation of DG/CDP-DG Synthesis—A major branch point in phospholipid synthesis involves the enzymes PA phosphatase and CDP-DG synthase. These enzymes utilize PA as a substrate (Fig. 1). The partitioning of PA at this step in the pathway would influence the levels of individual phospholipids and would also alter the proportions of the phospholipids and the neutral lipids, DG and TG. Based on the relative K_m values for PA, the 45- and 104-kDa forms of PA phosphatase have a greater affinity for PA than does CDP-DG synthase (34, 36, 37). This suggests that the partitioning of PA between CDP-DG and DG may be primarily governed by the regulation of PA phosphatase activity. The 45- and 104-kDa PA phosphatase activities are each inhibited by sphingoid bases (*i.e.* sphinganine and phytosphingosine) (93) and ATP (54). However, they are regulated differentially by phosphorylation (94). cAMP-dependent protein kinase phosphorylates and activates the 45-kDa enzyme but has no effect on the 104-kDa PA phosphatase (94). The regulation of PA phosphatase activity by sphingoid bases, ATP, and phosphorylation correlates with observed changes in the synthesis of phospholipids and TG (52, 54, 92, 94–96). Both PA phosphatase activities are activated by CL, CDP-DG, and PI (97). Since the activation constants for these phospholipids are within the range of their cellular concentrations (97), this activation may be physiologically relevant. In contrast to the PA phosphatases, CDP-DG synthase activity is not regulated by phosphorylation, nucleotides, sphingoid bases, or phospholipids (52, 98, 99).

Regulation of PS/PI Synthesis—A second branch point in phos-

TABLE II
Biochemical regulation of phospholipid biosynthetic enzymes

The table lists those enzymes, discussed in the text, that have been shown to be regulated by biochemical mechanisms.

Enzyme	Regulated by	Effect	Ref.
45-kDa PA phosphatase	cAMP-dependent protein kinase phosphorylation	Activation	94
	CL, CDP-DG, PI, DGPP	Activation	44, 97
	Sphingoid bases	Inhibition	93
	Nucleotides	Inhibition	54
104-kDa PA phosphatase	CL, CDP-DG, PI, DGPP	Activation	44, 97
	Sphingoid bases	Inhibition	93
	Nucleotides	Inhibition	54
PS synthase	cAMP-dependent protein kinase phosphorylation	Inhibition	94
	PA	Activation	101
	CL, DG	Inhibition	101
	Sphingoid bases	Inhibition	52
	Inositol	Inhibition	47
45-kDa PI 4-kinase	CDP-DG, PG	Inhibition	50
	Nucleotides	Inhibition	102
55-kDa PI 4-kinase	Nucleotides	Inhibition	102
IPC synthase	Sphingoid bases	Inhibition	52

pholipid biosynthesis involves the enzymes PS synthase and PI synthase. These enzymes both utilize CDP-DG as a substrate (Fig. 1). Data suggest that the partitioning of CDP-DG between PS and PI is primarily determined by the level of PS synthase activity. Inositol regulates the expression of PS synthase (64, 65) and regulates the activity of the enzyme by acting as a noncompetitive inhibitor (47). PS synthase activity is also inhibited by sphingoid bases (52) and by cAMP-dependent protein kinase phosphorylation (100). The inhibition of PS synthase activity by inositol (47), sphingoid bases (52), and phosphorylation (99) results in an increase in PI synthesis and a concomitant reduction in PS synthesis *in vivo*. PA, CL, and DG also regulate PS synthase activity (101). PA activates PS synthase activity while CL and DG inhibit its activity. The activation constants for these lipids are within the range of their cellular concentrations (101), which suggests that the regulation of PS synthase activity by these lipids may be physiologically relevant.

In contrast to PS synthase, PI synthase activity is not regulated by phospholipid precursors (47, 90), phospholipids (101), sphingoid bases (52), or phosphorylation (99). Data indicate that the partitioning of CDP-DG between PS and PI is not governed by the affinities that PI synthase and PS synthase have for CDP-DG (47). Given the low intracellular levels of inositol and the relative high K_m value for inositol, the synthesis of PI by PI synthase *in vivo* is likely to be regulated primarily by the availability of this substrate (47).

Regulation of Phosphoinositide/Sphingolipid Synthesis—PI is a branch point intermediate for the synthesis of the phosphoinositides (PIP, PIP_2, and PI 3-P) and sphingolipids (IPC, MIPC, and $M(IP)_2C$) (Fig. 1). Given this, the regulation of PI 4-kinase and IPC synthase activities could play a pivotal role in the partitioning of PI between these lipids. Two membrane-associated forms of PI 4-kinase (45 and 55 kDa) have been identified and characterized (40–42). Regulation of the 45- and 55-kDa PI 4-kinase activities by ATP and ADP plays a major role in the synthesis of PIP and PIP_2 *in vivo* (102). The activities of these PI 4-kinases are not regulated by cAMP-dependent protein kinase phosphorylation (102), a mechanism previously thought to regulate the membrane-associated forms of the enzyme (103, 104). The 45-kDa PI 4-kinase is inhibited by CDP-DG, and the inhibitor constant for the enzyme is within its plasma membrane concentration (50). Moreover, regulation of the 45-kDa PI 4-kinase activity by CDP-DG is coordinated with the regulation of enzymes in the CDP-DG pathway (50). IPC synthase has not been purified, and little is known about its biochemical regulation. However, studies with a solubilized preparation of the enzyme have shown that IPC synthase activity is inhibited by sphingoid bases and that this inhibition correlates with a decrease in sphingolipid synthesis (52).

Regulation of DG/PS Synthesis—The responses of PA phosphatase and PS synthase to various modulators further illustrate the reciprocal nature of the regulation of phospholipid synthesis. The DG generated from PA by the PA phosphatase can be used to synthesize TG and phospholipids by the Kennedy pathway, while PS synthase can use the CDP-DG derived from PA by the action of CDP-DG synthase (Fig. 1). Inositol supplementation elevates levels of the 45-kDa PA phosphatase (37) but reduces levels of the PS synthase (64, 65). Phosphorylation of the 45-kDa PA phosphatase by cAMP-dependent protein kinase stimulates its activity (94), while phosphorylation of PS synthase inhibits its activity (100). Both enzymes are regulated by phospholipids but in a complementary manner. PS synthase activity is activated by PA (101) while PA phosphatase activity is activated by CDP-DG (97). Thus, the phospholipid substrate for PA phosphatase activates PS synthase, while the phospholipid substrate for PS synthase activates PA phosphatase. In addition, DG (the product of the PA phosphatase reaction) inhibits PS synthase activity (101). Finally, CL activates PA phosphatase activity (97) but inhibits PS synthase activity (101). These results suggest that the differential regulation of PA phosphatase and PS synthase plays a central role in controlling the pathways by which phospholipids and neutral lipids are synthesized.

Novel Enzymes of Phospholipid Metabolism

DGPP phosphatase is a membrane-associated enzyme recently identified in *S. cerevisiae* (44). This enzyme catalyzes the dephosphorylation of DGPP to generate PA. DGPP is a novel metabolite that contains a pyrophosphate group attached to DG (105). This phospholipid accounts for 0.18 mol % of the total phospholipid content in *S. cerevisiae* (44). When DGPP is supplied as a substrate *in vitro*, the enzyme removes the β-phosphate of DGPP to generate PA and then removes the α-phosphate to generate DG (44). In fact, DGPP phosphatase can utilize PA as a substrate in the absence of DGPP, although the enzyme has a 10-fold higher specificity constant for DGPP (44). *In vitro*, the DGPP phosphatase activity of the enzyme is not significantly altered by PC, PE, PI, PS, or DG (44). In addition, PA does not alter DGPP phosphatase activity (44). However, DGPP does competitively inhibit the PA phosphatase activity of the DGPP phosphatase enzyme (44). In contrast, DGPP stimulates the activities of the 45- and 104-kDa PA phosphatases (44). Moreover, these PA phosphatase enzymes do not utilize DGPP as a substrate (44). These data indicate that the activities of the DGPP phosphatase enzyme and of the DGPP phospholipid may influence PA levels *in vivo*. Since PA plays a major role in phospholipid metabolism, it is likely that the activities of the DGPP phosphatase enzyme will influence these processes.

Another enzyme activity that has been recently identified in *S. cerevisiae* is PA kinase (44). This enzyme catalyzes the phosphorylation of PA to generate DGPP. The PA kinase from yeast has not been purified, and its response to various lipid regulators is not known. Since the activity of PA kinase and DGPP phosphatase will contribute to the levels of DGPP, PA, and DG, the enzymes likely participate in a novel cycle for the regulation of the levels of these lipids.

Concluding Comments

Research on phospholipid synthesis in *S. cerevisiae* has significantly advanced our understanding of this process. It is clear from studies of phospholipid enzymes and their genes that the mechanisms that govern this metabolism are intricate and are integrated with other aspects of cell physiology. Investigators are using a combination of approaches (genetic, molecular, and biochemical) to help resolve this complexity. This effort requires the cloning of those genes that encode enzymes that have been identified as well as the purification and characterization of the products of these genes. In addition, as the recent discovery of DGPP phosphatase and PA kinase illustrates, there may be other components of phospholipid metabolism yet to be identified.

Acknowledgments—We express our esteem and gratitude to the members of our laboratory, past and present, and to all of our colleagues who have contributed to the understanding of phospholipid biosynthesis in *S. cerevisiae*. We also acknowledge Susan A. Henry for help and encouragement throughout the course of our studies on phospholipid metabolism in yeast.

REFERENCES

1. Carman, G. M., and Henry, S. A. (1989) *Annu. Rev. Biochem.* **58,** 635–669
2. Paltauf, F., Kohlwein, S. D., and Henry, S. A. (1992) in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression* (Jones, E.

W., Pringle, J. R., and Broach, J. R., eds) pp. 415–500, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Shen, H., Heacock, P. N., Clancey, C. J., and Dowhan, W. (1996) *J. Biol. Chem.* **271,** 789–795
4. Letts, V. A., Klig, L. S., Bae-Lee, M., Carman, G. M., and Henry, S. A. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 7279–7283
5. Kiyono, K., Miura, K., Kushima, Y., Hikiji, T., Fukushima, M., Shibuya, I., and Ohta, A. (1987) *J. Biochem.* (*Tokyo*) **102,** 1089–1100
6. Nikawa, J., Tsukagoshi, Y., Kodaki, T., and Yamashita, S. (1987) *Eur. J. Biochem.* **167,** 7–12
7. Clancey, C. J., Chang, S.-C., and Dowhan, W. (1993) *J. Biol. Chem.* **268,** 24580–24590
8. Trotter, P. J., Pedretti, J., and Voelker, D. R. (1993) *J. Biol. Chem.* **268,** 21416–21424
9. Trotter, P. J., Pedretti, J., Yates, R., and Voelker, D. R. (1995) *J. Biol. Chem.* **270,** 6071–6080
10. Kodaki, T., and Yamashita, S. (1987) *J. Biol. Chem.* **262,** 15428–15435
11. Summers, E. F., Letts, V. A., McGraw, P., and Henry, S. A. (1988) *Genetics* **120,** 909–922
12. McGraw, P., and Henry, S. A. (1989) *Genetics* **122,** 317–330
13. Hjelmstad, R. H., and Bell, R. M. (1988) *J. Biol. Chem.* **263,** 19748–19757
14. Hjelmstad, R. H., and Bell, R. M. (1991) *J. Biol. Chem.* **266,** 5094–5103
15. Hosaka, K., Kodaki, T., and Yamashita, S. (1989) *J. Biol. Chem.* **264,** 2053–2059
16. Tsukagoshi, Y., Nikawa, J., and Yamashita, S. (1987) *Eur. J. Biochem.* **169,** 477–486
17. Hjelmstad, R. H., and Bell, R. M. (1987) *J. Biol. Chem.* **262,** 3909–3917
18. Hjelmstad, R. H., and Bell, R. M. (1990) *J. Biol. Chem.* **265,** 1755–1764
19. Klig, L. S., and Henry, S. A. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 3816–3820
20. Dean-Johnson, M., and Henry, S. A. (1989) *J. Biol. Chem.* **264,** 1274–1283
21. Nikawa, J., and Yamashita, S. (1984) *Eur. J. Biochem.* **143,** 251–256
22. Nikawa, J., Kodaki, T., and Yamashita, S. (1987) *J. Biol. Chem.* **262,** 4876–4881
23. Flanagan, C. A., Schnieders, E. S., Emerick, A. W., Kunisawa, R., Admon, A., and Thorner, J. (1993) *Science* **262,** 1444–1448
24. Herman, P. K., and Emr, S. D. (1990) *Mol. Cell. Biol.* **10,** 6742–6754
25. Schu, P. V., Takegawa, K., Fry, M. J., Stack, J. H., Waterfield, M. D., and Emr, S. D. (1993) *Science* **260,** 88–91
26. Atkinson, K. D., Jensen, B., Kolat, A. I., Storm, E. M., Henry, S. A., and Fogel, S. (1980) *J. Bacteriol.* **141,** 558–564
27. Nikawa, J., and Yamashita, S. (1981) *Biochim. Biophys. Acta* **665,** 420–426
28. Greenberg, M. L., Klig, L. S., Letts, V. A., Loewy, B. S., and Henry, S. A. (1983) *J. Bacteriol.* **153,** 791–799
29. Kodaki, T., and Yamashita, S. (1989) *Eur. J. Biochem.* **185,** 243–251
30. Nikawa, J., Yonemura, K., and Yamashita, S. (1983) *Eur. J. Biochem.* **131,** 223–229
31. Hosaka, K., and Yamashita, S. (1987) *Eur. J. Biochem.* **162,** 7–13
32. Culbertson, M. R., Donahue, T. F., and Henry, S. A. (1976) *J. Bacteriol.* **126,** 243–250
33. Nikawa, J., and Yamashita, S. (1982) *Eur. J. Biochem.* **125,** 445–451
34. Kelley, M. J., and Carman, G. M. (1987) *J. Biol. Chem.* **262,** 14563–14570
35. Bae-Lee, M., and Carman, G. M. (1984) *J. Biol. Chem.* **259,** 10857–10862
36. Lin, Y.-P., and Carman, G. M. (1989) *J. Biol. Chem.* **264,** 8641–8645
37. Morlock, K. R., McLaughlin, J. J., Lin, Y.-P., and Carman, G. M. (1991) *J. Biol. Chem.* **266,** 3586–3593
38. Donahue, T. F., and Henry, S. A. (1981) *J. Biol. Chem.* **256,** 7077–7085
39. Fischl, A. S., and Carman, G. M. (1983) *J. Bacteriol.* **154,** 304–311
40. Belunis, C. J., Bae-Lee, M., Kelley, M. J., and Carman, G. M. (1988) *J. Biol. Chem.* **263,** 18897–18903
41. Buxeda, R. J., Nickels, J. T., Jr., Belunis, C. J., and Carman, G. M. (1991) *J. Biol. Chem.* **266,** 13859–13865
42. Nickels, J. T., Jr., Buxeda, R. J., and Carman, G. M. (1992) *J. Biol. Chem.* **267,** 16297–16304
43. Flanagan, C. A., and Thorner, J. (1992) *J. Biol. Chem.* **267,** 24117–24125
44. Wu, W.-I., Liu, Y., Riedel, B., Wissing, J. B., Fischl, A. S., and Carman, G. M. (1996) *J. Biol. Chem.* **271,** 1868–1876
45. Greenberg, M. L., and Lopes, J. M. (1996) *Microbiol. Rev.* **60,** 1–20
46. Klig, L. S., Homann, M. J., Carman, G. M., and Henry, S. A. (1985) *J. Bacteriol.* **162,** 1135–1141
47. Kelley, M. J., Bailis, A. M., Henry, S. A., and Carman, G. M. (1988) *J. Biol. Chem.* **263,** 18078–18085
48. Becker, G. W., and Lester, R. L. (1977) *J. Biol. Chem.* **252,** 8684–8691
49. Henry, S. A., Atkinson, K. D., Kolat, A. J., and Culbertson, M. R. (1977) *J. Bacteriol.* **130,** 472–484
50. Nickels, J. T., Jr., Buxeda, R. J., and Carman, G. M. (1994) *J. Biol. Chem.* **269,** 11018–11024
51. Letts, V. A., and Henry, S. A. (1985) *J. Bacteriol.* **163,** 560–567
52. Wu, W.-I., McDonough, V. M., Nickels, J. T., Jr., Ko, J., Fischl, A. S., Vales, T. R., Merrill, A. H., Jr., and Carman, G. M. (1995) *J. Biol. Chem.* **270,** 13171–13178
53. Talwalkar, R. T., and Lester, R. L. (1973) *Biochim. Biophys. Acta* **306,** 412–421
54. Wu, W.-I., and Carman, G. M. (1994) *J. Biol. Chem.* **269,** 29495–29501
55. Kennedy, E. P., and Weiss, S. B. (1956) *J. Biol. Chem.* **222,** 193–214
56. Kennedy, E. P. (1986) in *Lipids and Membranes: Past, Present and Future* (Op den Kamp, J. A. F., Roelofsen, B., and Wirtz, K. W. A. eds) pp. 171–206, Elsevier Science Publishers B. V., Amsterdam
57. Henry, S. A. (1982) in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression* (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 101–158, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
58. Carman, G. M. (1989) in *Phosphatidylcholine Metabolism* (Vance, D. E., ed) pp. 165–183, CRC Press, Inc., Boca Raton, FL
59. Atkinson, K., Fogel, S., and Henry, S. A. (1980) *J. Biol. Chem.* **255,** 6653–6661
60. Trotter, P. J., and Voelker, D. R. (1995) *J. Biol. Chem.* **270,** 6062–6070
61. McDonough, V. M., Buxeda, R. J., Bruno, M. E. C., Ozier-Kalogeropoulos, O., Adeline, M.-T., McMaster, C. R., Bell, R. M., and Carman, G. M. (1995) *J. Biol. Chem.* **270,** 18774–18780
62. Homann, M. J., Henry, S. A., and Carman, G. M. (1985) *J. Bacteriol.* **163,** 1265–1266
63. Klig, L. S., Homann, M. J., Kohlwein, S., Kelley, M. J., Henry, S. A., and Carman, G. M. (1988) *J. Bacteriol.* **170,** 1878–1886
64. Poole, M. A., Homann, M. J., Bae-Lee, M., and Carman, G. M. (1986) *J. Bacteriol.* **168,** 668–672
65. Bailis, A. M., Poole, M. A., Carman, G. M., and Henry, S. A. (1987) *Mol. Cell. Biol.* **7,** 167–176
66. Carson, M. A., Atkinson, K. D., and Waechter, C. J. (1982) *J. Biol. Chem.* **257,** 8115–8121
67. Carson, M. A., Emala, M., Hogsten, P., and Waechter, C. J. (1984) *J. Biol. Chem.* **259,** 6267–6273
68. Overmeyer, J. H., and Waechter, C. J. (1991) *Arch. Biochem. Biophys.* **290,** 511–516
69. Lamping, E., Kohlwein, S. D., Henry, S. A., and Paltauf, F. (1991) *J. Bacteriol.* **173,** 6432–6437
70. Gaynor, P. M., Gill, T., Toutenhoofd, S., Summers, E. F., McGraw, P., Homann, M. J., Henry, S. A., and Carman, G. M. (1991) *Biochim. Biophys. Acta* **1090,** 326–332
71. Yamashita, S., Oshima, A., Nikawa, J., and Hosaka, K. (1982) *Eur. J. Biochem.* **128,** 589–595
72. Yamashita, S., and Oshima, A. (1980) *Eur. J. Biochem.* **104,** 611–616
73. Waechter, C. J., and Lester, R. L. (1973) *Arch. Biochem. Biophys.* **158,** 401–410
74. Morash, S. C., McMaster, C. R., Hjelmstad, R. H., and Bell, R. M. (1994) *J. Biol. Chem.* **269,** 28769–28776
75. McGee, T. P., Skinner, H. B., Whitters, E. A., Henry, S. A., and Bankaitis, V. A. (1994) *J. Cell Biol.* **124,** 273–287
76. McMaster, C. R., and Bell, R. M. (1994) *J. Biol. Chem.* **269,** 28010–28016
77. Hosaka, K., Murakami, T., Kodaki, T., Nikawa, J., and Yamashita, S. (1990) *J. Bacteriol.* **172,** 2005–2012
78. McMaster, C. R., and Bell, R. M. (1994) *J. Biol. Chem.* **269,** 14776–14783
79. Nikawa, J., Hosaka, K., and Yamashita, S. (1993) *Mol. Microbiol.* **10,** 955–961
80. Lai, K., and McGraw, P. (1994) *J. Biol. Chem.* **269,** 2245–2251
81. Nikawa, J., Hosaka, K., Tsukagoshi, Y., and Yamashita, S. (1990) *J. Biol. Chem.* **265,** 15996–16003
82. Hirsch, J. P., and Henry, S. A. (1986) *Mol. Cell. Biol.* **6,** 3320–3328
83. Greenberg, M., Goldwasser, P., and Henry, S. A. (1982) *Mol. & Gen. Genet.* **186,** 157–163
84. Greenberg, M., Reiner, B., and Henry, S. A. (1982) *Genetics* **100,** 19–33
85. Ambroziak, J., and Henry, S. A. (1994) *J. Biol. Chem.* **269,** 15344–15349
86. Nikoloff, D. M., and Henry, S. A. (1994) *J. Biol. Chem.* **269,** 7402–7411
87. Bachhawat, N., Ouyang, Q., and Henry, S. A. (1995) *J. Biol. Chem.* **270,** 25087–25095
88. Lopes, J. M., Hirsch, J. P., Chorgo, P. A., Schulze, K. L., and Henry, S. A. (1991) *Nucleic Acids Res.* **19,** 1687–1693
89. Bailis, A. M., Lopes, J. M., Kohlwein, S. D., and Henry, S. A. (1992) *Nucleic Acids Res.* **20,** 1411–1418
90. Fischl, A. S., Homann, M. J., Poole, M. A., and Carman, G. M. (1986) *J. Biol. Chem.* **261,** 3178–3183
91. Ko, J., Cheah, S., and Fischl, A. S. (1994) *J. Bacteriol.* **176,** 5181–5183
92. Morlock, K. R., Lin, Y.-P., and Carman, G. M. (1988) *J. Bacteriol.* **170,** 3561–3566
93. Wu, W., Lin, Y.-P., Wang, E., Merrill, A. H., Jr., and Carman, G. M. (1993) *J. Biol. Chem.* **268,** 13830–13837
94. Quinlan, J. J., Nickels, J. T., Jr., Wu, W., Lin, Y.-P., Broach, J. R., and Carman, G. M. (1992) *J. Biol. Chem.* **267,** 18013–18020
95. Hosaka, K., and Yamashita, S. (1984) *Biochim. Biophys. Acta* **796,** 110–117
96. Taylor, F. R., and Parks, L. W. (1979) *Biochim. Biophys. Acta* **575,** 204–214
97. Wu, W.-I., and Carman, G. M. (1996) *Biochemistry* **35,** 3790–3796
98. Kelley, M. J. (1989) *Purification and Characterization of CDP-diacylglycerol Synthase from Yeast. Regulation of CDP-diacylglycerol-dependent Enzymes by Inositol.* Ph.D. thesis, Rutgers University
99. Kinney, A. J., Bae-Lee, M., Singh Panghaal, S., Kelley, M. J., Gaynor, P. M., and Carman, G. M. (1990) *J. Bacteriol.* **172,** 1133–1136
100. Kinney, A. J., and Carman, G. M. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 7962–7966
101. Bae-Lee, M., and Carman, G. M. (1990) *J. Biol. Chem.* **265,** 7221–7226
102. Buxeda, R. J., Nickels, J. T., Jr., and Carman, G. M. (1993) *J. Biol. Chem.* **268,** 6248–6255
103. Holland, K. M., Homann, M. J., Belunis, C. J., and Carman, G. M. (1988) *J. Bacteriol.* **170,** 828–833
104. Kato, H., Uno, I., Ishikawa, T., and Takenawa, T. (1989) *J. Biol. Chem.* **264,** 3116–3121
105. Wissing, J. B., and Behrbohm, H. (1993) *FEBS Lett.* **315,** 95–99