# Phosphatidic Acid Plays a Central Role in the Transcriptional Regulation of Glycerophospholipid Synthesis in *Saccharomyces cerevisiae*[*]

**George M. Carman**[‡1] **and Susan A. Henry**[§]

*From the ‡Department of Food Science and the Rutgers Center for Lipid Research, Rutgers University, New Brunswick, New Jersey 08901 and the §Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853*

In eukaryotic cells, PA[2] is a central precursor for the synthesis of major glycerophospholipids, DAG and TAG, as well as a major signaling lipid. In mammalian cells, PA is implicated as an activator of cell growth and proliferation, vesicular trafficking, secretion, and endocytosis (1–7). In plants, PA is implicated in seed germination and response to stress induced by drought, salinity, and low temperature (3, 4).

The signaling roles of PA in the yeast *Saccharomyces cerevisiae* have not received as much attention as they have in higher eukaryotic cells. However, it is known that PA production via phospholipase D-mediated turnover of PC is necessary for suppression of growth and membrane trafficking defects in mutants defective in Sec14p, an essential PI/PC-binding protein (8–10). In addition, PA production via phospholipase D is implicated in Spo20p-mediated fusion of vesicles with the prespore membrane during sporogenesis (11, 12). The best studied regulatory function of PA in *S. cerevisiae* is its role as a signaling molecule in the transcriptional regulation of glycerophospholipid synthesis itself, the major topic of this review. We will focus on the pathways generating and utilizing PA in yeast and the evidence that PA plays a central role in the transcriptional regulation of glycerophospholipid synthesis.

## The Role of PA in de Novo Lipid Synthesis

PA is the precursor of all membrane glycerophospholipids and the storage lipid TAG in *S. cerevisiae* (Fig. 1) (13–16). In the *de novo* pathway, PA is synthesized from glycerol 3-phosphate and then used for the synthesis of glycerophospholipids via the liponucleotide intermediate CDP-DAG (*i.e.* CDP-DAG pathway) (Fig. 1). PA is also used for the synthesis of PE and PC via DAG in the Kennedy pathway (CDP-ethanolamine and CDP-

choline branches, respectively) (Fig. 1). The DAG derived from PA is also used for the synthesis of TAG (Fig. 1). Cells grown in the absence of choline synthesize PC primarily via the CDP-DAG pathway (17). In contrast, cells supplemented with choline synthesize PC primarily via the Kennedy pathway (17). Nonetheless, PC is synthesized from PA by both the CDP-DAG and Kennedy pathways regardless of whether choline is added to the growth medium (8, 18–22). The choline required is derived from the phospholipase D-mediated turnover of the PC synthesized via the CDP-DAG pathway (Fig. 1) (8, 10). The PA produced is reincorporated into glycerophospholipids (8).

Mutants defective in PC synthesis via the CDP-DAG pathway require choline for growth; they synthesize PC via the CDP-choline branch of the Kennedy pathway (23–30). Mutants defective in the synthesis of PS (23, 24) and PE (25, 26) can synthesize PC if they are supplemented with ethanolamine. The ethanolamine is used to synthesize PE via the CDP-ethanolamine branch of the Kennedy pathway, which is then methylated to form PC in the CDP-DAG pathway (Fig. 1) (31, 32). Kennedy pathway mutants (*e.g. cki1 eki1* and *cpt1 ept1*) defective in both the CDP-choline and CDP-ethanolamine branches must synthesize PC via the CDP-DAG pathway (18, 20, 33). However, unlike mutants defective in the CDP-DAG pathway (23–30), Kennedy pathway mutants do not exhibit any auxotrophic requirements (18, 33).

## Inositol and Nutrient Depletion Regulate the Expression of UAS<sub>INO</sub>-containing Glycerophospholipid Synthesis Genes

The synthesis of PC is coordinately regulated with the synthesis of PI (31, 32, 34, 35). This regulation is mediated by genetic and biochemical mechanisms (31, 32, 34–37). In this review, we will focus on the transcriptional regulation of glycerophospholipid synthesis gene expression. Several glycerophospholipid synthesis genes are maximally expressed when inositol is absent from the growth medium and repressed when inositol is added to the growth medium. The repression by inositol requires PC synthesis and is enhanced by the inclusion of choline in the growth medium (15, 31, 32, 38, 39). The expression of glycerophospholipid synthesis genes is also repressed when cells enter the stationary phase of growth and when cells are depleted of nitrogen or zinc (31, 32, 35). The repression in the stationary phase and by nitrogen and zinc depletion occurs in the absence of inositol (31, 32, 35, 40).

The coordinate regulation of the glycerophospholipid synthesis genes by inositol supplementation, growth phase, and nutrient depletion is dependent on the transcriptional regulatory proteins Ino2p (41), Ino4p (42), and Opi1p (43), as well as a UAS<sub>INO</sub> *cis*-acting element (44–47) present in the promoters of the glycerophospholipid synthesis genes (Fig. 2) (31, 32, 39, 48). The UAS<sub>INO</sub> element contains a consensus binding site (CATGTGAAAT) for an Ino2p-Ino4p heterodimer complex that activates the expression of glycerophospholipid synthesis

2 The abbreviations used are: PA, phosphatidic acid; DAG, diacylglycerol; TAG, triacylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; UAS<sub>INO</sub>, upstream activating sequence inositol-responsive element; ER, endoplasmic reticulum.
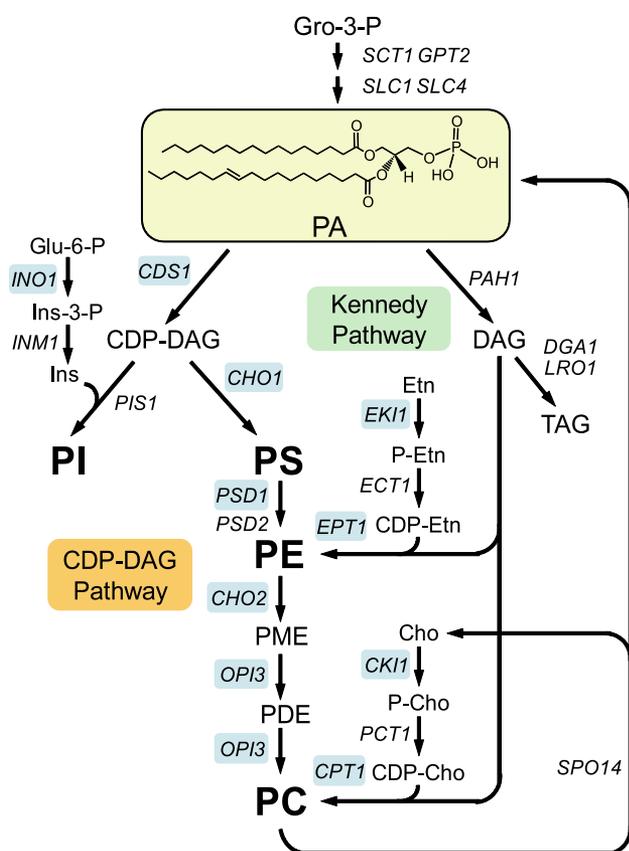
FIGURE 1. **Structure of PA and its role in lipid synthesis.** The structure of PA is shown with fatty acyl groups of 16:0 (*sn*-1) and 18:1 (*sn*-2). The pathways shown for the synthesis of glycerophospholipids include the relevant steps discussed in this review. A more comprehensive pathway of lipid metabolism may be found in Ref. 34. The genes that encode the enzymes catalyzing individual steps in the pathway are indicated. The UAS$_{INO}$-containing genes that are subject to regulation by inositol supplementation and to nutrient depletion are highlighted in *blue*. *Gro*, glycerol; *Ins*, inositol; *PME*, phosphatidylmonomethylethanolamine; *PDE*, phosphatidyldimethylethanolamine; *Etn*, ethanolamine; *P-Etn*, phosphoethanolamine; *Cho*, choline; *P-Cho*, phosphocholine.



FIGURE 2. **Model for the role of PA in the transcriptional regulation of UAS$_{INO}$-containing genes.** *A*, UAS$_{INO}$-containing genes (*blue*) are maximally expressed (*thick arrow*) when wild-type *S. cerevisiae* cells are grown in the absence of inositol and in the presence of sufficient amounts of essential nutrients. *B*, when inositol is added to the growth medium or when cells are depleted of an essential nutrient, transcription of UAS$_{INO}$-containing genes is attenuated (*thin arrow*) by the interaction of Opi1p with Ino2p. Dissociation of Opi1p from the nuclear/ER membrane is caused by a decrease in PA concentration, which is mediated by the stimulation of PI synthesis and by activation of Mg$^{2+}$-dependent PA phosphatase (*PAP1*, denoted by the *star*). *Etn*, ethanolamine; *Cho*, choline; *Ins*, inositol.

genes (31, 32, 39, 49–52). Repression of these genes is dependent on Opi1p (43, 53), which inhibits transcriptional activation of the Ino2p-Ino4p complex by binding to Ino2p (Fig. 2) (54). In fact, *ino2* and *ino4* mutants exhibit constitutively repressed levels of UAS$_{INO}$-containing genes, whereas *opi1* mutants exhibit constitutively derepressed levels of UAS$_{INO}$-containing genes (15, 31, 38, 39). Because of the misregulation of *INO1* expression, *ino2* and *ino4* mutants are auxotrophic for inositol, whereas *opi1* mutants produce excessive amounts of inositol and excrete it into the growth medium (15, 31, 38, 39). Thus, inositol auxotrophy and inositol excretion are used as indicators of the misregulation of *INO1* and other UAS$_{INO}$-containing genes (15, 31, 38, 39).

Recent genome-wide studies using microarray analysis have revealed the full extent of genes responsive to addition of inositol and choline to the growth medium and dependent for this regulation on Ino2p, Ino4p, and Opi1p (55–57). These studies also revealed several other major classes of genes in addition to and independent of Ino2p, Ino4p, and Opi1p that respond to signals arising in the ER following inositol addition that are potentially related to lipid metabolism (57).
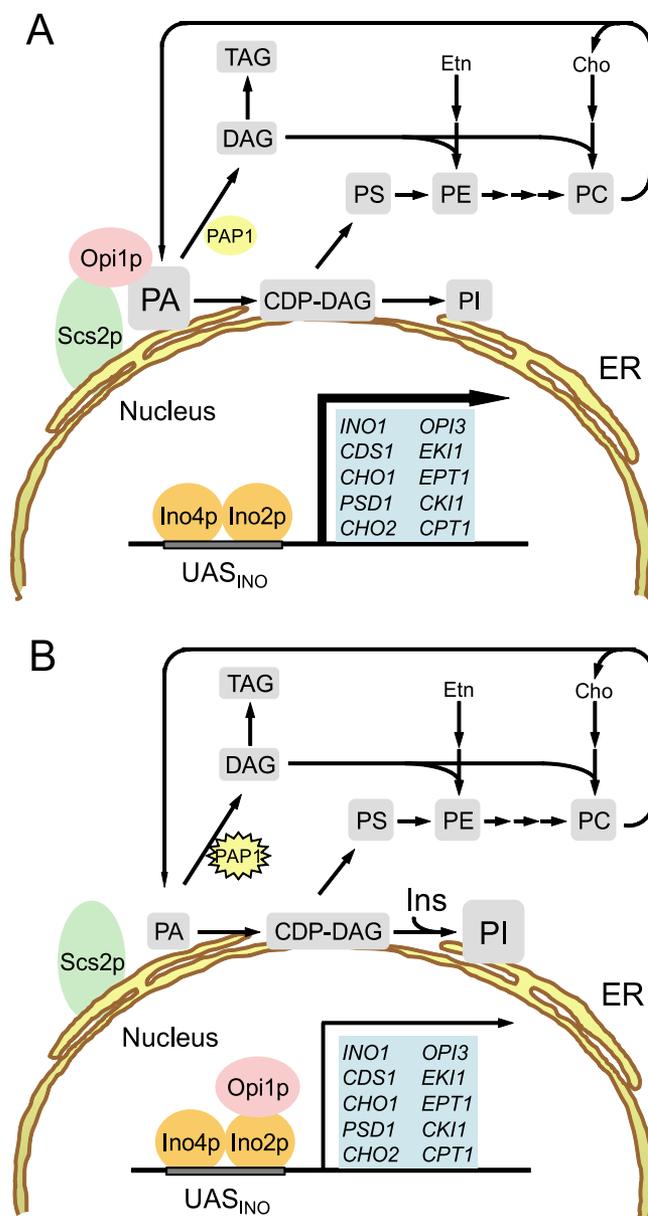
## Genetic Clues Implicating PA as the Signaling Molecule That Regulates Transcription of UAS$_{INO}$-containing Glycerophospholipid Synthesis Genes

Mutations that block the utilization of PA for PC synthesis cause the derepression of *INO1* and other UAS$_{INO}$-containing genes, whereas mutations that increase the utilization of PA cause the repression of UAS$_{INO}$-containing genes (31, 32). Mutants defective in the structural genes leading to PC from PA via the CDP-DAG pathway (Fig. 1) share in common the inosi-

tol excretion phenotype (31, 39). As indicated above, the inositol excretion phenotype results from the loss of Opi1p repressor function, which leads to the constitutive derepression and overexpression of *INO1*. Moreover, the remaining wild-type genes in a particular CDP-DAG pathway mutant cannot be repressed by inositol supplementation (31, 32). The inositol excretion phenotype of the CDP-DAG pathway mutants beginning with the formation of PS from CDP-DAG can be complemented if the mutants are supplemented with a water-soluble precursor that allows for the synthesis of glycerophospholipids via the Kennedy pathway (31, 32). The supplementation of the Kennedy pathway precursor also permits the inositol-mediated repression of the remaining wild-type genes in a particular CDP-DAG pathway mutant (31, 32). A complete block of the first step in the CDP-DAG pathway (*e.g. cds1*) is lethal (58). However, partial (59) or conditional (60) *cds1* alleles derepress *INO1* and excrete inositol (60). The inositol excretion phenotype of the *cds1* mutants cannot be overcome by supplementation with a Kennedy pathway precursor, nor will inositol mediate the repression of other UAS$_{INO}$-containing genes in these strains (31, 32, 59). Thus, mutants defective at every step in the synthesis of PS, PE, and PC from CDP-DAG exhibit increases in PA levels and altered regulation of *INO1* and have the inositol excretion phenotype. These observations led to the hypothesis that PA is the metabolic signal for *INO1* derepression (31).

In contrast, mutants defective in PC synthesis via the CDP-choline branch of the Kennedy pathway do not excrete inositol and exhibit normal regulation of *INO1* (61). The Kennedy pathway mutants do, however, exhibit a choline excretion phenotype (8). Interestingly, Kennedy pathway mutants that also possess a mutation in the *SEC14* gene (*e.g. cki1 sec14*, *pct1 sec14*, and *cpt1 sec14*) exhibit extreme choline excretion in combination with an inositol excretion phenotype (8, 9). The excretion of choline and inositol by these double mutants is dependent on the phospholipase D-mediated turnover of PC (8, 9). The finding that excess PA produced by this reaction causes *INO1* derepression even in the presence of inositol (8, 9) further supports the hypothesis that PA provides the signal for derepression of UAS$_{INO}$-containing genes (31).

The enzyme responsible for generating the DAG for glycerophospholipid synthesis via the Kennedy pathway is the *PAH1*-encoded Mg$^{2+}$-dependent PA phosphatase (62). Mutations that affect the activity of this enzyme cause the misregulation of UAS$_{INO}$-containing genes (62–64). A *pah1* mutant with reduced activity exhibits derepressed levels of *INO1*, *OPI3*, and *INO2* (62, 63). However, the level of *INO1* derepression is not sufficient to cause inositol excretion (62, 63). This may be the consequence of the significant amount of Mg$^{2+}$-dependent PA phosphatase activity that remains in *pah1* mutant cells (62). Pah1p is phosphorylated by cyclin-dependent Cdc28p kinase and dephosphorylated by the Nem1p-Spo7p phosphatase complex (63). Reduction in Pah1p phosphorylation elevates Mg$^{2+}$-dependent PA phosphatase activity (64), indicating that phosphorylation inhibits activity. Cells that overexpress a phosphorylation-deficient enzyme are repressed for *INO1* and *OPI3* and require inositol for growth (64). The fact that the inositol auxotrophy of cells overexpressing Mg$^{2+}$-dependent

PA phosphatase activity can be suppressed by an *opi1* mutation (64) indicates the role of Opi1p in this regulation.

## PA Content Controls the Localization of Opi1p and Its Repressor Function

Loewen *et al.* (65– 67) have shown that the Opi1p repressor, a protein lacking membrane-spanning domains (43), is found at the nuclear/ER membrane and within the nucleus. Opi1p associates with the nuclear/ER membrane through interaction with the integral membrane protein Scs2p (Fig. 2) (65). A connection between Scs2p and the regulation of glycerophospholipid synthesis is evident because *scs2* mutants are auxotrophic for inositol due to the constitutive repression of *INO1* (68–70). Thus, attenuation of the repressor activity of Opi1p depends on its association with the nuclear/ER membrane through interaction with Scs2p. Interestingly, the *scs2* mutation also results in an increase in PC synthesis via the Kennedy pathway, and a block in the Kennedy pathway (*e.g. cki1 scs2*) restores normal *INO1* expression (70). As discussed above, PC synthesis via the Kennedy pathway consumes PA via DAG, and a block in the Kennedy pathway should accumulate PA, thus potentially explaining the suppression of the inositol auxotrophy of *scs2* mutants. Although this scenario has not been fully explored experimentally, it is consistent with the notion that PA plays a role in the regulation of *INO1* and other UAS$_{INO}$-containing genes.

A major breakthrough in our understanding of glycerophospholipid synthesis regulation was the discovery that Opi1p is a PA-binding protein and that its association with the nuclear/ER membrane is stabilized by PA (66). Moreover, Loewen *et al.* (66) have shown that Opi1p translocates from the nuclear/ER membrane into the nucleus upon inositol supplementation, which results in a reduction in the level of PA coincident with its use as a precursor in the synthesis of PI via CDP-DAG (Fig. 2). The translocation of Opi1p in response to inositol supplementation coincides with the repression of *INO1* expression (66). Thus, a key regulatory switch for controlling the expression of *INO1* and other UAS$_{INO}$-containing genes is the localization of Opi1p, which is dependent on the presence of a certain amount of PA that is required for stabilizing the association of Opi1p at the nuclear/ER membrane (66). The establishment of this mechanism thus provided confirmation of the signaling role of PA predicted by the genetic experiments described above (31). Changes in PA concentration can be brought about by the action and regulation of several enzymes (*e.g.* CDP-DAG pathway enzymes, Kennedy pathway enzymes, Mg$^{2+}$-dependent PA phosphatase, and phospholipase D) and thus are predicted to affect the location and repressor activity of Opi1p. As discussed below, this also explains why the expression of UAS$_{INO}$-containing genes is regulated by growth phase and nutrient depletion (*e.g.* nitrogen and zinc), conditions that lower PA levels, independent of inositol availability.

## Model for the Transcriptional Regulation of UAS$_{INO}$-containing Genes

A model for the regulation of UAS$_{INO}$-containing genes by inositol and by nutrient depletion is presented in Fig. 2. According to this model, Opi1p is associated with the nuclear/ER

membrane through interactions with PA and Scs2p when exponential phase cells are grown without inositol and in the presence of sufficient amounts of essential nutrients (*e.g.* zinc) (Fig. 2*A*). Upon inositol supplementation, exponential phase cells synthesize an elevated level of PI through increased substrate availability (71) and draw upon the pool of PA at the nuclear/ER membrane (Fig. 2*B*) (66). At the same time, inositol supplementation results in an increase in $Mg^{2+}$-dependent PA phosphatase activity (72), which contributes to the decrease in the PA pool. TAG is synthesized at the expense of glycerophospholipids in stationary phase cells (73), and $Mg^{2+}$-dependent PA phosphatase activity is elevated under this growth condition (74). The *PAH1*-encoded $Mg^{2+}$-dependent PA phosphatase enzyme plays a major role in TAG synthesis, and its increased activity in stationary phase diminishes the PA pool (62). In zinc-depleted cells, PI synthesis increases (75), and this condition should draw upon the PA pool. The increase in PI synthesis in response to zinc depletion is not the result of elevated levels of inositol; *INO1* is actually repressed under this growth condition (75). Instead, the increase in PI synthesis is due to the Zap1p-mediated induction of *PIS1* gene expression (76). Furthermore, zinc depletion induces $Mg^{2+}$-dependent PA phosphatase activity (77), and this would further reduce the PA pool. Thus, regardless of whether cells are supplemented with inositol or are depleted of an essential nutrient, a decrease in the PA level results in loss of Opi1p association with the nuclear/ER membrane, followed by Opi1p translocation into the nucleus. In the nucleus, Opi1p mediates repression of the $UAS_{INO}$-containing glycerophospholipid synthesis genes that are co-regulated through its interaction with Ino2p.

## Perspectives

The control of enzymes (*e.g.* CDP-DAG synthase, $Mg^{2+}$-dependent PA phosphatase, and phospholipase D) that directly contribute to the PA pool at the nuclear/ER membrane should have a major impact on glycerophospholipid synthesis regulation by Opi1p. As indicated above, the phosphorylation state of $Mg^{2+}$-dependent PA phosphatase plays a role in the Opi1p-mediated regulation of *INO1* and *OPI3* (64). However, it is unclear how various growth conditions (*e.g.* inositol supplementation and zinc depletion) affect the phosphorylation-dephosphorylation of the enzyme. Opi1p is phosphorylated by protein kinase A (78), protein kinase C (79), and casein kinase II (80). Phosphorylation by protein kinase A and casein kinase II stimulates Opi1p repressor function (78, 80), whereas phosphorylation by protein kinase C attenuates Opi1p repressor function (79). Whether these phosphorylation events influence the interaction of Opi1p with PA, Scs2p, or Ino2p is unclear.

Inositol auxotrophy has been described in conjunction with mutations in several major signaling pathways that include the unfolded protein response (81–83), the protein kinase C/mitogen-activated protein kinase (MAPK) pathway (84), and the glucose response pathway (85). These observations have led to the speculation that these signaling pathways might play a direct role in transmitting the signal controlling *INO1* expression. The demonstration that Opi1p translocation into the nucleus following inositol supplementation in response to PA availability is the major signal for repression of $UAS_{INO}$-con-

taining genes indicates that these signaling pathways must play a more peripheral role. Such roles could include modulation of Opi1p activity by phosphorylation, as suggested for protein kinase C (79), or by influencing histone phosphorylation and subsequent acetylation at the *INO1* locus, as suggested for the glucose response pathway (86, 87). However, effects on histone modification at *INO1* cannot fully explain the inositol auxotrophy of *snf1* and *snf4* mutants defective in the glucose response pathway because these phenotypes can be suppressed by direct inhibition of the enzymatic activity of acetyl-CoA carboxylase (85), the enzyme controlling the rate-limiting step in fatty acid synthesis. Acetyl-CoA carboxylase is a direct target of Snf1p (related to mammalian AMP-activated protein kinase) in both yeast and mammals (88). Thus, additional signals coming from fatty acid synthesis may also influence *INO1* expression. It is quite possible that these signals are also coordinated through the *de novo* synthesis of PA because acyl-CoAs serve as immediate precursors. Future work on the coordination of lipid metabolism with other cellular processes, such as membrane trafficking and responses to stress and nutrient availability, via the major signaling pathways should provide new insights into the myriad roles of PA in the cell. These insights should provide models of metabolic regulation and lipid signaling testable in both animals and plants.

## REFERENCES

1. Waggoner, D. W., Xu, J., Singh, I., Jasinska, R., Zhang, Q. X., and Brindley, D. N. (1999) *Biochim. Biophys. Acta* **1439,** 299–316
2. Sciorra, V. A., and Morris, A. J. (2002) *Biochim. Biophys. Acta* **1582,** 45–51
3. Testerink, C., and Munnik, T. (2005) *Trends Plant Sci.* **10,** 368–375
4. Wang, X., Devaiah, S. P., Zhang, W., and Welti, R. (2006) *Prog. Lipid Res.* **45,** 250–278
5. Brindley, D. N. (2004) *J. Cell. Biochem.* **92,** 900–912
6. Howe, A. G., and McMaster, C. R. (2006) *Can. J. Physiol. Pharmacol.* **84,** 29–38
7. Foster, D. A. (2007) *Cancer Res.* **67,** 1–4
8. Patton-Vogt, J. L., Griac, P., Sreenivas, A., Bruno, V., Dowd, S., Swede, M. J., and Henry, S. A. (1997) *J. Biol. Chem.* **272,** 20873–20883
9. Sreenivas, A., Patton-Vogt, J. L., Bruno, V., Griac, P., and Henry, S. A. (1998) *J. Biol. Chem.* **273,** 16635–16638
10. Xie, Z. G., Fang, M., Rivas, M. P., Faulkner, A. J., Sternweis, P. C., Engebrecht, J., and Bankaitis, V. A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 12346–12351
11. Rudge, S. A., Sciorra, V. A., Iwamoto, M., Zhou, C., Strahl, T., Morris, A. J., Thorner, J., and Engebrecht, J. (2004) *Mol. Biol. Cell* **15,** 207–218
12. Nakanishi, H., Morishita, M., Schwartz, C. L., Coluccio, A., Engebrecht, J., and Neiman, A. M. (2006) *J. Cell Sci.* **119,** 1406–1415
13. Rattray, J. B., Schibeci, A., and Kidby, D. K. (1975) *Bacteriol. Rev.* **39,** 197–231
14. 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
15. 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

16. Czabany, T., Athenstaedt, K., and Daum, G. (2007) *Biochim. Biophys. Acta* **1771,** 299–309
17. McMaster, C. R., and Bell, R. M. (1994) *J. Biol. Chem.* **269,** 14776–14783
18. Morash, S. C., McMaster, C. R., Hjelmstad, R. H., and Bell, R. M. (1994) *J. Biol. Chem.* **269,** 28769–28776
19. McGee, T. P., Skinner, H. B., Whitters, E. A., Henry, S. A., and Bankaitis, V. A. (1994) *J. Cell Biol.* **124,** 273–287
20. McMaster, C. R., and Bell, R. M. (1994) *J. Biol. Chem.* **269,** 28010–28016
21. Ostrander, D. B., O'Brien, D. J., Gorman, J. A., and Carman, G. M. (1998) *J. Biol. Chem.* **273,** 18992–19001
22. Kim, K.-H., Voelker, D. R., Flocco, M. T., and Carman, G. M. (1998) *J. Biol. Chem.* **273,** 6844–6852
23. Atkinson, K., Fogel, S., and Henry, S. A. (1980) *J. Biol. Chem.* **255,** 6653–6661
24. Atkinson, K. D., Jensen, B., Kolat, A. I., Storm, E. M., Henry, S. A., and Fogel, S. (1980) *J. Bacteriol.* **141,** 558–564
25. Trotter, P. J., and Voelker, D. R. (1995) *J. Biol. Chem.* **270,** 6062–6070
26. Trotter, P. J., Pedretti, J., Yates, R., and Voelker, D. R. (1995) *J. Biol. Chem.* **270,** 6071–6080
27. Kodaki, T., and Yamashita, S. (1987) *J. Biol. Chem.* **262,** 15428–15435
28. Kodaki, T., and Yamashita, S. (1989) *Eur. J. Biochem.* **185,** 243–251
29. Summers, E. F., Letts, V. A., McGraw, P., and Henry, S. A. (1988) *Genetics* **120,** 909–922
30. McGraw, P., and Henry, S. A. (1989) *Genetics* **122,** 317–330
31. Henry, S. A., and Patton-Vogt, J. L. (1998) *Prog. Nucleic Acid Res.* **61,** 133–179
32. Carman, G. M., and Henry, S. A. (1999) *Prog. Lipid Res.* **38,** 361–399
33. Kim, K., Kim, K.-H., Storey, M. K., Voelker, D. R., and Carman, G. M. (1999) *J. Biol. Chem.* **274,** 14857–14866
34. Gaspar, M. L., Aregullin, M. A., Jesch, S. A., Nunez, L. R., Villa-Garcia, M., and Henry, S. A. (2007) *Biochim. Biophys. Acta* **1771,** 241–254
35. Carman, G. M., and Han, G.-S. (2007) *Biochim. Biophys. Acta* **1771,** 322–330
36. Carman, G. M., and Zeimetz, G. M. (1996) *J. Biol. Chem.* **271,** 13293–13296
37. Carman, G. M., and Kersting, M. C. (2004) *Biochem. Cell Biol.* **82,** 62–70
38. Carman, G. M., and Henry, S. A. (1989) *Annu. Rev. Biochem.* **58,** 635–669
39. Greenberg, M. L., and Lopes, J. M. (1996) *Microbiol. Rev.* **60,** 1–20
40. Griac, P., and Henry, S. A. (1999) *Nucleic Acids Res.* **27,** 2043–2050
41. Nikoloff, D. M., McGraw, P., and Henry, S. A. (1992) *Nucleic Acids Res.* **20,** 3253
42. Hoshizaki, D. K., Hill, J. E., and Henry, S. A. (1990) *J. Biol. Chem.* **265,** 4736–4745
43. White, M. J., Hirsch, J. P., and Henry, S. A. (1991) *J. Biol. Chem.* **266,** 863–872
44. Kodaki, T., Nikawa, J., Hosaka, K., and Yamashita, S. (1991) *J. Bacteriol.* **173,** 7992–7995
45. Lopes, J. M., Hirsch, J. P., Chorgo, P. A., Schulze, K. L., and Henry, S. A. (1991) *Nucleic Acids Res.* **19,** 1687–1693
46. Schuller, H. J., Hahn, A., Troster, F., Schutz, A., and Schweizer, E. (1992) *EMBO J.* **11,** 107–114
47. Schuller, H. J., Richter, K., Hoffmann, B., Ebbert, R., and Schweizer, E. (1995) *FEBS Lett.* **370,** 149–152
48. Ambroziak, J., and Henry, S. A. (1994) *J. Biol. Chem.* **269,** 15344–15349
49. Hirsch, J. P., and Henry, S. A. (1986) *Mol. Cell. Biol.* **6,** 3320–3328
50. Bachhawat, N., Ouyang, Q., and Henry, S. A. (1995) *J. Biol. Chem.* **270,** 25087–25095
51. Loewy, B. S., and Henry, S. A. (1984) *Mol. Cell. Biol.* **4,** 2479–2485
52. Schwank, S., Ebbert, R., Rautenstrauss, K., Schweizer, E., and Schuller, H. J. (1995) *Nucleic Acids Res.* **23,** 230–237
53. Greenberg, M., Reiner, B., and Henry, S. A. (1982) *Genetics* **100,** 19–33
54. Wagner, C., Dietz, M., Wittmann, J., Albrecht, A., and Schuller, H. J. (2001) *Mol. Microbiol.* **41,** 155–166
55. Santiago, T. C., and Mamoun, C. B. (2003) *J. Biol. Chem.* **278,** 38723–38730
56. Jesch, S. A., Zhao, X., Wells, M. T., and Henry, S. A. (2005) *J. Biol. Chem.* **280,** 9106–9118
57. Jesch, S. A., Liu, P., Zhao, X., Wells, M. T., and Henry, S. A. (2006) *J. Biol. Chem.* **281,** 24070–24083
58. Shen, H., Heacock, P. N., Clancey, C. J., and Dowhan, W. (1996) *J. Biol. Chem.* **271,** 789–795
59. Klig, L. S., Homann, M. J., Kohlwein, S. D., Kelley, M. J., Henry, S. A., and Carman, G. M. (1988) *J. Bacteriol.* **170,** 1878–1886
60. Shen, H., and Dowhan, W. (1996) *J. Biol. Chem.* **271,** 29043–29048
61. Griac, P., Swede, M. J., and Henry, S. A. (1996) *J. Biol. Chem.* **271,** 25692–25698
62. Han, G.-S., Wu, W.-I., and Carman, G. M. (2006) *J. Biol. Chem.* **281,** 9210–9218
63. Santos-Rosa, H., Leung, J., Grimsey, N., Peak-Chew, S., and Siniossoglou, S. (2005) *EMBO J.* **24,** 1931–1941
64. O'Hara, L., Han, G.-S., Peak-Chew, S., Grimsey, N., Carman, G. M., and Siniossoglou, S. (2006) *J. Biol. Chem.* **281,** 34537–34548
65. Loewen, C. J. R., Roy, A., and Levine, T. P. (2003) *EMBO J.* **22,** 2025–2035
66. Loewen, C. J. R., Gaspar, M. L., Jesch, S. A., Delon, C., Ktistakis, N. T., Henry, S. A., and Levine, T. P. (2004) *Science* **304,** 1644–1647
67. Loewen, C. J. R., and Levine, T. P. (2005) *J. Biol. Chem.* **280,** 14097–14104
68. Nikawa, J., Murakami, A., Esumi, E., and Hosaka, K. (1995) *J. Biochem. (Tokyo)* **118,** 39–45
69. Kagiwada, S., Hosaka, K., Murata, M., Nikawa, J., and Takatsuki, A. (1998) *J. Bacteriol.* **180,** 1700–1708
70. Kagiwada, S., and Zen, R. (2003) *J. Biochem. (Tokyo)* **133,** 515–522
71. Kelley, M. J., Bailis, A. M., Henry, S. A., and Carman, G. M. (1988) *J. Biol. Chem.* **263,** 18078–18085
72. Morlock, K. R., Lin, Y.-P., and Carman, G. M. (1988) *J. Bacteriol.* **170,** 3561–3566
73. Taylor, F. R., and Parks, L. W. (1979) *Biochim. Biophys. Acta* **575,** 204–214
74. Hosaka, K., and Yamashita, S. (1984) *Biochim. Biophys. Acta* **796,** 110–117
75. Iwanyshyn, W. M., Han, G.-S., and Carman, G. M. (2004) *J. Biol. Chem.* **279,** 21976–21983
76. Han, S.-H., Han, G.-S., Iwanyshyn, W. M., and Carman, G. M. (2005) *J. Biol. Chem.* **280,** 29017–29024
77. Iwanyshyn, W. M. (2005) *Regulation of Phospholipid Synthesis in Saccharomyces cerevisiae by Zinc.* Ph.D. dissertation, Rutgers University, New Brunswick, NJ
78. Sreenivas, A., and Carman, G. M. (2003) *J. Biol. Chem.* **278,** 20673–20680
79. Sreenivas, A., Villa-Garcia, M. J., Henry, S. A., and Carman, G. M. (2001) *J. Biol. Chem.* **276,** 29915–29923
80. Chang, Y.-F., and Carman, G. M. (2006) *J. Biol. Chem.* **281,** 4754–4761
81. Nikawa, J., and Yamashita, S. (1992) *Mol. Microbiol.* **6,** 1441–1446
82. Cox, J. S., Shamu, C. E., and Walter, P. (1993) *Cell* **73,** 1197–1206
83. Chang, H. J., Jesch, S. A., Gaspar, M. L., and Henry, S. A. (2004) *Genetics* **168,** 1899–1913
84. Nunez, L. R. (2006) *Phospholipid Synthesis in Yeast: The Role of the PKC1-MPK1 Signal Transduction Pathway.* Ph.D. dissertation, Cornell University, Ithaca, NY
85. Shirra, M. K., Patton-Vogt, J., Ulrich, A., Liuta-Tehlivets, O., Kohlwein, S. D., Henry, S. A., and Arndt, K. M. (2001) *Mol. Cell. Biol.* **21,** 5710–5722
86. Lo, W. S., Duggan, L., Emre, N. C., Belotserkovskya, R., Lane, W. S., Shiekhattar, R., and Berger, S. L. (2001) *Science* **293,** 1142–1146
87. Lo, W. S., Gamache, E. R., Henry, K. W., Yang, D., Pillus, L., and Berger, S. L. (2005) *EMBO J.* **24,** 997–1008
88. Woods, A., Munday, M. R., Scott, J., Yang, X., Carlson, M., and Carling, D. (1994) *J. Biol. Chem.* **269,** 19509–19515