Regulation of phospholipid synthesis in yeast by zinc

G.M. Carman[1]
Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, U.S.A.

Abstract

The yeast *Saccharomyces cerevisiae* has the ability to cope with a variety of stress conditions (e.g. zinc deficiency) by regulating the expression of enzyme activities including those involved with phospholipid synthesis. Zinc is an essential mineral required for the growth and metabolism of *S. cerevisiae*. Depletion of zinc from the growth medium of wild-type cells results in alterations in phospholipid composition including an increase in PI (phosphatidylinositol) and a decrease in phosphatidylethanolamine. These changes can be attributed to an increase in *PIS1*-encoded PI synthase activity and a decrease in the activities of several CDP-diacylglycerol pathway enzymes including the *CHO1*-encoded PS (phosphatidylserine) synthase. The reduction in PS synthase in response to zinc depletion is due to a repression mechanism that involves the UAS_{INO} (inositol upstream activating sequence) element in the *CHO1* promoter and the negative transcription factor Opi1p. These factors are also responsible for the inositol-mediated repression of *CHO1*. This regulation may play an important role in allowing cells to adapt to zinc deficiency given the essential roles that phospholipids play in the structure and function of cellular membranes.

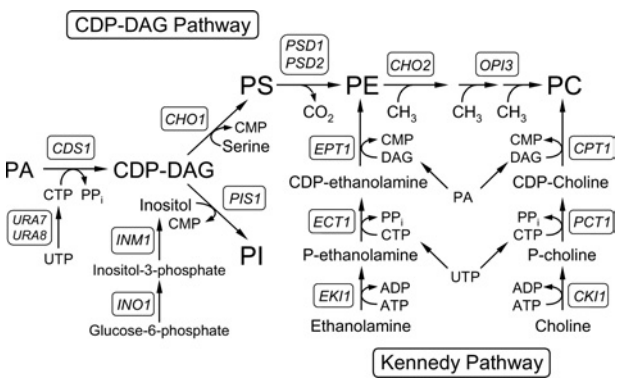
Phospholipids are amphipathic molecules that are essential for vital cellular processes. In addition to being a major structural component of cellular membranes, they serve in protein modification for membrane association [1], as molecular chaperones [2] and also as reservoirs of lipid second messengers [3]. PC (phosphatidylcholine), PE (phosphatidylethanolamine), PI (phosphatidylinositol) and PS (phosphatidylserine) are the major phospholipids found in the cellular membranes of eukaryotic cells [4]. Phosphatidylglycerol and cardiolipin are also found in mitochondrial membranes [4]. The yeast *Saccharomyces cerevisiae* is a model eukaryote being used to study the regulation of phospholipid synthesis [5,6]. The synthesis of phospholipids in *S. cerevisiae* (Scheme 1), which occurs by pathways common to those found in mammalian cells [4], is regulated by growth phase, phospholipid precursors, phosphorylation of enzymes and transcription factors, and as will be discussed in this review, by zinc availability [4–10].

Zinc is an essential mineral needed for growth and metabolism of yeast and higher eukaryotes [11]. The essential nature of zinc stems from the fact that it is a cofactor for hundreds of enzymes and a structural constituent of many proteins [11,12]. In rats, zinc deficiency is associated with oxidative damage to DNA, proteins and lipids [13]. Zinc deficiency in humans is manifested by defects in appetite, cognitive function, embryonic development, epithelial integrity and immune function [14]. The underlining effect of zinc deficiency is on cell division and differentiation [11]. Notwithstanding, zinc is toxic if it accumulates in excessive quantities [11]. Our interest in the regulation of phospholipid synthesis by zinc originates from the observation that depletion of zinc from the growth medium of yeast results in the Zap1p-mediated induction of the *DPP1* gene and its product DGPP (diacylglycerol pyrophosphate) phosphatase [8]. Zap1p is a zinc-sensing and zinc-inducible transcription factor that binds to a UAS_{ZRE} (where UAS stands for upstream activating sequence and ZRE for zinc-responsive element) found in the promoter of many zinc-regulated genes to activate their transcription [15–18]. In fact, *DPP1* is one of the most highly regulated genes that respond to zinc depletion in the *S. cerevisiae* genome [18]. The *DPP1*-encoded DGPP phosphatase is a vacuole membrane-associated enzyme that catalyses the removal of the β-phosphate from DGPP to form PA (phosphatidate), and it then removes the phosphate from PA to form diacylglycerol [8,19]. Indeed, the regulation of DGPP phosphatase expression correlates with the metabolism of DGPP and PA in the vacuolar membranes [20]. When grown in the presence of zinc, DGPP and PA account for 0.6 and 1.4 mol% of the total phospholipids in vacuolar membranes. Depletion of zinc from the growth medium results in a decrease in DGPP to an undetectable level and a decrease in PA to 0.3 mol% [20]. The *DPP1* gene is not essential, and *dpp1*Δ mutant cells do not exhibit any dramatic phenotypes under a variety of growth conditions [21], including fluctuations in zinc supplementation [22]. Thus the role of DGPP phosphatase during zinc depletion would have to complement other mechanisms that respond to this stress. Although the function of DGPP phosphatase in yeast is still unclear, we speculate that it functions to control the levels of DGPP and PA in vacuolar membranes, which in

Key words: inositol, phosphatidylinositol, phosphatidylserine, phospholipid, yeast, zinc.
Abbreviations used: CDP-DAG, CDP-diacylglycerol; DGPP, diacylglycerol pyrophosphate; ER, endoplasmic reticulum; PA, phosphatidate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; UAS_{INO}, inositol upstream activating sequence; ZRE, zinc-responsive element.
[1]email carman@aesop.rutgers.edu

Scheme 1 | **Pathways for the synthesis of phospholipids in *S. cerevisiae***

The pathways shown for the synthesis of phospholipids include the relevant steps discussed in the review. The CDP-DAG and Kennedy pathways and the known genes that encode enzymes catalysing individual steps in the pathway are indicated.



turn mediates other cellular functions that occur in response to stress.

In addition to the changes in DGPP and PA, zinc depletion results in a reduction in the level of PE and an increase in the level of PI in the vacuole membrane [20]. Analysis of *dpp1*Δ mutant cells depleted for zinc indicates that the alterations in the major vacuole membrane phospholipids are not dependent on the regulation of *DPP1* expression and DGPP phosphatase activity [23]. Thus the effects of zinc depletion on phospholipid synthesis are more global. In fact, zinc depletion results in alterations in the cellular levels of the major membrane phospholipids PE and PI [9] similar to that observed in the vacuole membranes [20]. Analysis of a plasma membrane zinc transport mutant indicates that changes in phospholipid composition are dependent on the cytosolic levels of zinc [9].

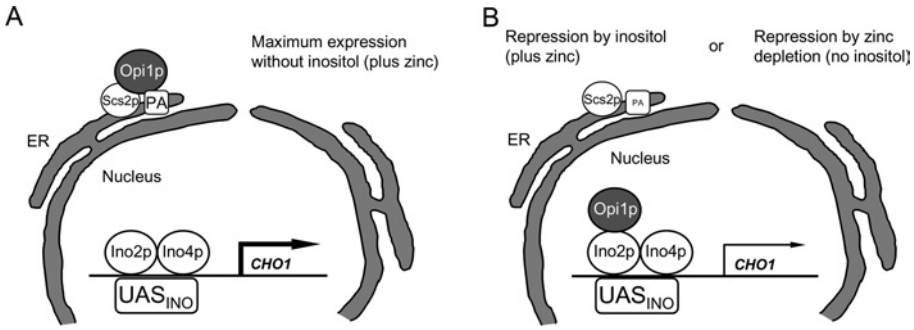
The effects of zinc depletion on the expression of several phospholipid biosynthetic enzyme activities have been examined in wild-type cells. When cells are grown without inositol and choline, the major membrane phospholipids are primarily synthesized via the CDP-DAG (diacylglycerol) pathway (Scheme 1) [6]. In this pathway, PS, PE and PC are synthesized from CDP-DAG via the reactions catalysed by *CHO1*-encoded PS synthase [24–26], *PSD1*/*PSD2*-encoded PS decarboxylase [27–29], *CHO2*-encoded PE methyltransferase [30,31] and *OPI3*-encoded phospholipid methyltransferase [30,32]. Zinc-depleted cells show reduced activity levels (50, 25, 50 and 36% respectively) of these CDP-DAG pathway enzymes [9]. On the other hand, the activity of *PIS1*-encoded PI synthase [33], which competes with PS synthase for the substrate CDP-DAG [34], is elevated by 2-fold in zinc-depleted cells [9]. The decrease in PE content correlates with the decreases in the activities of PS synthase and PS decarboxylase, while the increase in PI content correlates with the increase in the activity of PI synthase. Thus the regulation of phospholipid synthesis contributes to alterations in phospholipid composition. Although the activities of the phospholipid methyltransferase enzymes are reduced in zinc-depleted cells, this growth condition does not have a major effect on PC content [9]. Enzymes in the CDP-choline branch of the Kennedy pathway for PC synthesis (Scheme 1) might be activated to compensate for the decrease in activities of the CDP-DAG pathway enzymes. Additional studies are needed to address this hypothesis.

The co-ordinate regulation of the PI synthase and PS synthase enzymes, which compete for CDP-DAG as a substrate (Scheme 1), is part of an overall mechanism by which the synthesis of PI is co-ordinately regulated with the synthesis of PC [5,6]. Given the importance of these two enzymes in phospholipid synthesis, the mechanisms responsible for their regulation by zinc depletion are being examined. Preliminary studies presented at the Seventh Yeast Lipid Conference in Swansea (U.K.) indicate that the elevated expression of PI synthase activity in response to zinc depletion is due to the induction of the *PIS1* gene. Studies are in progress to substantiate this conclusion. Studies on the regulation of the *CHO1*-encoded PS synthase in response to zinc depletion are further along.

Results from Northern-blot analysis and an analysis of β-galactosidase activity driven by a P_{CHO1}-*lacZ* reporter gene have shown that zinc depletion leads to the repression of *CHO1* transcription, and this result correlates with decreased levels of the PS synthase protein and activity [9]. The lack of a UAS_{ZRE} in the promoter of the *CHO1* gene indicates that the transcription factor Zap1p does not directly regulate PS synthase expression in response to zinc depletion. Moreover, an indirect effect of Zap1p on the expression of PS synthase is ruled out because the *zap1*Δ mutation does not affect the zinc-mediated regulation of the enzyme [9]. Instead, the expression of PS synthase by zinc is controlled through the UAS_{INO} (inositol UAS) element in the *CHO1* promoter and by the transcription factors Ino2p, Ino4p and Opi1p [9]. This conclusion is based on the fact that mutations in the UAS_{INO} element in the *CHO1* promoter and mutations in the transcription factors Ino2p, Ino4p and Opi1p eliminate the repression of *CHO1* expression by zinc depletion [9]. This is an unexpected finding since these regulatory components play an important role in the inositol-mediated regulation of *CHO1* and other UAS_{INO}-containing genes (e.g. *INO1*) involved in phospholipid synthesis [5,6,35]. In fact, the *INO1* gene is also repressed when cells are deprived of zinc [9]. Interestingly, the repression of the *CHO1* and *INO1* genes in response to zinc depletion occurs in the absence of inositol supplementation [9].

Inositol is the precursor to PI and is synthesized in *S. cerevisiae* via the *INO1*-encoded inositol-3-phosphate synthase (Scheme 1). Inositol plays a major role in the regulation of phospholipid synthesis in yeast. The inositol-mediated regulation of *CHO1* and other UAS_{INO}-containing genes involved in phospholipid synthesis has been extensively characterized [5,6,35]. A model for the regulation of *CHO1*, which is based on a recent paper by Loewen et al. [36], is presented in Figure 1. The *CHO1* gene is maximally

Figure 1 | **Models for the regulation of *CHO1* by inositol supplementation or by zinc depletion**



expressed when inositol is absent from the growth medium, and it is repressed when inositol is supplemented to the growth medium. The repression of *CHO1* by inositol is enhanced by the inclusion of choline in the growth medium [5,6,35]. Inositol-mediated regulation of *CHO1* involves the UAS$_{INO}$ element in its promoter and the transcription factors Ino2p, Ino4p and Opi1p [5,6,35]. Ino2p [37] and Ino4p [38] are positive transcriptional regulators whereas Opi1p [39] is a negative transcriptional regulator. The UAS$_{INO}$ element contains a binding site for an Ino2p–Ino4p heterodimer, which is required for maximum expression (indicated by a thick arrow in Figure 1A) of the *CHO1* gene [5,6,35]. Opi1p is associated with the ER (endoplasmic reticulum) through interactions with the integral membrane protein Scs2p [a VAP (vesicle-associated membrane-protein-associated protein) homologue] [40] and with PA [36] when cells are grown without inositol. Upon inositol addition, the level of PA reduces due to the utilization of CDP-DAG and increased synthesis of PI [36]. The decrease in PA results in loss of Opi1p association with the ER and its translocation into the nucleus [36]. Opi1p represses transcription through the UAS$_{INO}$ element [41], but not by direct interaction [42]. Results indicate that Opi1p represses transcriptional activation (indicated by a thin arrow in Figure 1B) by binding to DNA-bound Ino2p [43]. In addition, the global repressor Sin3p interacts with the N-terminal region of Opi1p [43], and this interaction plays some role in Opi1p repressor function [42]. We propose that the model for the Opi1p-mediated repression of *CHO1* by inositol supplementation may be applicable to its regulation by zinc depletion (Figure 1). This hypothesis is supported by the observation that zinc depletion does not lead to a further repression of *CHO1* when gene expression is already reduced by inositol supplementation [9].

S. cerevisiae has the ability to cope with a variety of stress conditions by regulating the expression of enzymes including those involved in phospholipid synthesis. As discussed in this review, PI synthase and the enzyme activities in the CDP-DAG pathway are regulated by the stress condition of zinc depletion. This must play an important role in allowing the cells to adapt to zinc deficiency given the essential roles that phospholipids play in the structure and function of membranes.

G.-S. Han is acknowledged for helpful discussions.

References

1 Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Kominami, E., Ohsumi, M. et al. (2000) Nature (London) **408**, 488–492
2 Bogdanov, M. and Dowhan, W. (1999) J. Biol. Chem. **274**, 36827–36830
3 Exton, J.H. (1994) Biochim. Biophys. Acta **1212**, 26–42
4 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 Press, Plainview, NY
5 Henry, S.A. and Patton-Vogt, J.L. (1998) Prog. Nucleic Acid Res. **61**, 133–179
6 Carman, G.M. and Henry, S.A. (1999) Prog. Lipid Res. **38**, 361–399
7 Carman, G.M. and Zeimetz, G.M. (1996) J. Biol. Chem. **271**, 13293–13296
8 Han, G.-S., Johnston, C.N., Chen, X., Athenstaedt, K., Daum, G. and Carman, G.M. (2001) J. Biol. Chem. **276**, 10126–10133
9 Iwanyshyn, W.M., Han, G.S. and Carman, G.M. (2004) J. Biol. Chem. **279**, 21976–21983
10 Carman, G.M. and Kersting, M.C. (2004) Biochem. Cell. Biol. **82**, 62–70
11 Vallee, B.L. and Falchuk, K.H. (1993) Physiol. Rev. **73**, 79–118
12 Schwabe, J.W. and Klug, A. (1994) Nat. Struct. Biol. **1**, 345–349
13 Oteiza, P.L., Olin, K.L., Fraga, C.G. and Keen, C.L. (1996) Proc. Soc. Exp. Biol. Med. **213**, 85–91
14 Walsh, C.T., Sandstead, H.H., Prasad, A.S., Newberne, P.M. and Fraker, P.J. (1994) Environ. Health Perspect. **102**, 5–46
15 Zhao, H. and Eide, D.J. (1997) Mol. Cell. Biol. **17**, 5044–5052
16 Zhao, H., Butler, E., Rodgers, J., Spizzo, T., Duesterhoeft, S. and Eide, D. (1998) J. Biol. Chem. **273**, 28713–28720
17 Bird, A.J., McCall, K., Kramer, M., Blankman, E., Winge, D.R. and Eide, D.J. (2003) EMBO J. **22**, 5137–5146
18 Lyons, T.J., Gasch, A.P., Gaither, L.A., Botstein, D., Brown, P.O. and Eide, D.J. (2000) Proc. Natl. Acad. Sci. U.S.A. **97**, 7957–7962
19 Wu, W.-I., Liu, Y., Riedel, B., Wissing, J.B., Fischl, A.S. and Carman, G.M. (1996) J. Biol. Chem. **271**, 1868–1876
20 Han, G.-S., Johnston, C.N. and Carman, G.M. (2004) J. Biol. Chem. **279**, 5338–5345
21 Toke, D.A., Bennett, W.L., Dillon, D.A., Chen, X., Oshiro, J., Ostrander, D.B., Wu, W.-I., Cremesti, A., Voelker, D.R., Fischl, A.S. et al. (1998) J. Biol. Chem. **273**, 3278–3284
22 Yuan, D.S. (2000) Genetics **156**, 45–58
23 Johnston, C.N. (2002) M.S. thesis, Rutgers University, New Brunswick, NJ
24 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

25 Nikawa, J., Tsukagoshi, Y., Kodaki, T. and Yamashita, S. (1987) Eur. J. Biochem. **167**, 7–12
26 Kiyono, K., Miura, K., Kushima, Y., Hikiji, T., Fukushima, M., Shibuya, I. and Ohta, A. (1987) J. Biochem. (Tokyo) **102**, 1089–1100
27 Clancey, C.J., Chang, S.-C. and Dowhan, W. (1993) J. Biol. Chem. **268**, 24580–24590
28 Trotter, P.J., Pedretti, J. and Voelker, D.R. (1993) J. Biol. Chem. **268**, 21416–21424
29 Trotter, P.J., Pedretti, J., Yates, R. and Voelker, D.R. (1995) J. Biol. Chem. **270**, 6071–6080
30 Kodaki, T. and Yamashita, S. (1987) J. Biol. Chem. **262**, 15428–15435
31 Summers, E.F., Letts, V.A., McGraw, P. and Henry, S.A. (1988) Genetics **120**, 909–922
32 McGraw, P. and Henry, S.A. (1989) Genetics **122**, 317–330
33 Nikawa, J., Kodaki, T. and Yamashita, S. (1987) J. Biol. Chem. **262**, 4876–4881
34 Nikawa, J. and Yamashita, S. (1984) Eur. J. Biochem. **143**, 251–256
35 Greenberg, M.L. and Lopes, J.M. (1996) Microbiol. Rev. **60**, 1–20
36 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
37 Nikoloff, D.M., McGraw, P. and Henry, S.A. (1992) Nucleic Acids Res. **20**, 3253
38 Hoshizaki, D.K., Hill, J.E. and Henry, S.A. (1990) J. Biol. Chem. **265**, 4736–4745
39 White, M.J., Hirsch, J.P. and Henry, S.A. (1991) J. Biol. Chem. **266**, 863–872
40 Loewen, C.J., Roy, A. and Levine, T.P. (2003) EMBO J. **22**, 2025–2035
41 Bachhawat, N., Ouyang, Q. and Henry, S.A. (1995) J. Biol. Chem. **270**, 25087–25095
42 Wagner, C., Blank, M., Strohmann, B. and Schüller, H.J. (1999) Yeast **15**, 843–854
43 Wagner, C., Dietz, M., Wittmann, J., Albrecht, A. and Schuller, H.J. (2001) Mol. Microbiol. **41**, 155–166

Received 6 May 2005