MINIREVIEW / MINISYNTHÈSE

Phosphorylation synthesis in yeast: regulation by phosphorylation[1,2]

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Abstract: The yeast *Saccharomyces cerevisiae* is a model eukaryotic organism for the study of the regulation of phospholipid synthesis. The major phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine) are synthesized by complementary (CDP–diacylglycerol and Kennedy) pathways. The regulation of these pathways is complex and is controlled by genetic and biochemical mechanisms. Inositol plays a major role in the regulation of phospholipid synthesis. Inositol-mediated regulation involves the expression of genes and the modulation of enzyme activities. Phosphorylation is a major mechanism by which enzymes and transcription factors are regulated, and indeed, key phospholipid biosynthetic enzymes have been identified as targets of phosphorylation. Protein kinase A phosphorylates CTP synthetase, choline kinase, Mg^{2+}-dependent phosphatidate phosphatase, phosphatidylserine synthase, and the transcription factor Opi1p. CTP synthetase and Opi1p are also phosphorylated by protein kinase C. The phosphorylation of these proteins plays a role in regulating their activities and (or) function in phospholipid synthesis.

Key words: phospholipids, yeast, phospholipid synthesis, phosphorylation, protein kinase A, protein kinase C.

Résumé : La levure *Saccharomyces cerevisiae* est un modèle d'organisme eucaryote servant à étudier la régulation de la synthèse des phospholipides. Les principaux phospholipides (phosphatidylcholine, phosphatidyléthanolamine, phosphatidylinositol et phosphatidylsérine) sont synthétisés par des voies métaboliques complémentaires (CDP–diacylglycérol et Kennedy). La régulation de ces voies métaboliques, dans laquelle des mécanismes génétiques et biochimiques interviennent, est complexe. L'inositol joue un rôle crucial dans la régulation de la synthèse des phospholipides. L'expression de gènes et la modulation d'activités enzymatiques interviennent dans la régulation par l'intermédiaire de l'inositol. La phosphorylation est un mécanisme important de régulation d'enzymes et de facteurs de transcription et, en effet, des enzymes clés de la biosynthèse des phospholipides sont des cibles de la phosphorylation. La protéine kinase A phosphoryle la CTP synthétase, la choline kinase, la phosphatase de l'acide phosphatidique dépendante du Mg^{2+} et la phosphatidylsérine synthase, ainsi que le facteur de transcription Opi1p. La CTP synthétase et le facteur Opi1p sont également phosphorylés par la protéine kinase C. La phosphorylation de ces protéines joue un rôle dans la régulation de leurs activités et/ou de leur fonction dans la synthèse des phospholipides.

Mots clés : phospholipides, levure, synthèse des phospholipides, phosphorylation, protéine kinase A, protéine kinase C.

[Traduit par la Rédaction]

Introduction

The yeast *Saccharomyces cerevisiae* serves as a model eukaryotic organism for the study of the regulation of phospholipid synthesis (Carman and Henry 1989, 1999; Carman and Zeimetz 1996; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al. 1992). Almost all of the structural genes responsible for phospholipid synthesis in *S. cerevisiae* have been cloned and characterized, and mutations in these genes have been isolated (Carman and Henry 1989, 1999; Carman and Zeimetz 1996; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al. 1992; Zaremberg and McMaster 2002; Zheng and Zou 2001). In addition, many of the enzymes responsible for the synthesis of phospholipids have been purified and characterized (Carman and Henry 1989, 1999; Carman and Zeimetz

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1996; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al.1992). The characterization of the wild-type and mutant genes, along with their encoded enzymes, has significantly advanced the understanding of phospholipid synthesis and its regulation.

A number of factors regulate phospholipid synthesis in *S. cerevisiae*. These include water-soluble phospholipid precursors, nucleotides, lipids, and growth phase (Carman and Henry 1989, 1999; Carman and Zeimetz 1996; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al. 1992). Phospholipid synthesis regulation is complex and occurs by genetic and biochemical mechanisms. Moreover, regulation of phospholipid synthesis is interrelated with other cellular processes, including the metabolism of sphingolipids and triacylglycerols and general nutrient control (Carman and Henry 1999). In this review, we will discuss the pathways by which phospholipids are synthesized and their regulation by inositol. In addition, we will discuss recent advances in the regulation of phospholipid synthesis by phosphorylation.

Phospholipid biosynthetic pathways in *Saccharomyces cerevisiae*

Phospholipids are essential molecules that contribute to the structure and function of cell membranes. The major phospholipids found in the membranes of *S. cerevisiae* include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) (Carman and Henry 1989, 1999; Carman and Zeimetz 1996; Paltauf et al. 1992; Rattray et al. 1975). Mitochondrial membranes also contain phosphatidylglycerol and cardiolipin (Carman and Henry 1989, 1999; Carman and Zeimetz 1996; Paltauf et al. 1992; Rattray et al. 1975). The most common fatty acids esterified to the glycerophosphate backbone of yeast phospholipids include palmitic acid, palmitoleic acid, stearic acid, and oleic acid (Bossie and Martin 1989; McDonough et al.1992; Rattray et al. 1975). The synthesis of phospholipids in *S. cerevisiae* occurs by complementary pathways common to those found in mammalian cells[4] (Fig. 1) (Birner and Daum 2003; Carman and Henry 1989, 1999; Paltauf et al. 1992; Voelker 2003). PS, PE, and PC are synthesized from phosphatidate (PA) by the CDP–diacylglycerol (DAG) pathway (Fig. 1). The *CDS1*-encoded CDP–DAG synthase (Shen et al. 1996) catalyzes the formation of the energy-rich liponucleotide intermediate CDP–DAG from PA and CTP (Carter and Kennedy 1966). CDP–DAG may then donate its phosphatidyl moiety to serine to form PS (Kanfer and Kennedy 1964). This reaction is catalyzed by the *PSS1*/*CHO1*-encoded PS synthase (Kiyono et al. 1987; Letts et al. 1983; Nikawa et al. 1987*b*). PE is synthesized from PS by the reaction catalyzed by the *PSD1*-encoded (Clancey et al. 1993; Trotter et al. 1993) and *PSD2*-encoded (Trotter et al. 1995) PS decarboxylase enzymes. Two enzymes catalyze the three-step AdoMet-dependent methylation of PE to PC (Bremer and Greenberg 1961). The first methylation reaction is catalyzed by the *PEM1*/*CHO2*-encoded PE methyltransferase (Kodaki and Yamashita 1987; Summers et al. 1988) and the last two methylation reactions are catalyzed by the *PEM2*/*OPI3*-encoded phospholipid methyltransferase (Kodaki and Yamashita 1987; McGraw and Henry 1989).

PE and PC are also synthesized by way of the CDP–ethanolamine and CDP–choline branches, respectively, of the Kennedy pathway (Fig. 1). The *EKI1*-encoded ethanolamine kinase (Kim et al. 1999) and the *CKI1*-encoded choline kinase (Hosaka et al. 1989) enzymes catalyze the phosphorylations of ethanolamine and choline with ATP to form phosphoethanolamine and phosphocholine, respectively. The *ECT1*-encoded phosphoethanolamine cytidylyltransferase (Min-Seok et al. 1996) and the *PCT1*/*CCT1*-encoded phosphocholine cytidylyltransferase (Tsukagoshi et al. 1987) enzymes activate phosphoethanolamine and phosphocholine with CTP to form CDP–ethanolamine and CDP–choline, respectively. CDP–ethanolamine and CDP–choline react with DAG to form PE and PC, respectively, by reactions catalyzed by the *EPT1*-encoded ethanolamine phosphotransferase (Hjelmstad and Bell 1988, 1991) and the *CPT1*-encoded choline phosphotransferase (Hjelmstad and Bell 1987, 1990) enzymes.

The *PIS1*-encoded PI synthase (Nikawa et al. 1987*a*; Nikawa and Yamashita 1984) catalyzes the formation of PI by displacing CMP from CDP–DAG with inositol (Paulus and Kennedy 1960). The inositol used for this reaction is derived from glucose-6-phosphate via the reactions catalyzed by the *INO1*-encoded inositol-1-phosphate synthase (Dean-Johnson and Henry 1989; Klig and Henry 1984) and the *INM1*-encoded inositol-1-phosphate phosphatase (Murray and Greenberg 2000). CDP–DAG is also used for the synthesis of cardiolipin. In the cardiolipin pathway (not shown in Fig. 1), the *PGS1*-encoded phosphatidylglycerophosphate synthase (Chang et al. 1998*a*) displaces CMP from CDP–DAG with glycerol-3-phosphate to form phosphatidylglycerophosphate. This molecule is dephosphorylated by a phosphatase to form phosphatidylglycerol. The *CRD1*/*CLS1*-encoded cardiolipin synthase (Chang et al. 1998*b*; Jiang et al. 1997; Tuller et al. 1998) generates cardiolipin by catalyzing a reaction between phosphatidylglycerol and CDP–DAG.

The CTP required for the synthesis of the activated, energy-rich phospholipid pathway intermediates CDP–DAG, CDP–ethanolamine, and CDP–choline is derived from UTP via the reaction catalyzed by the *URA7*-encoded (Ozier-Kalogeropoulos et al. 1991) and *URA8*-encoded (Ozier-Kalogeropoulos et al. 1994) CTP synthetase enzymes. The DAG used for the synthesis of PE and PC via the Kennedy pathway is derived from PA by the reaction catalyzed by Mg^{2+}-dependent PA phosphatase (Carman 1997). DAG is also used for the synthesis of triacylglycerols (Carman 1997).

The CDP–DAG pathway is primarily used by wild-type cells for the synthesis of PE and PC when they are grown in the absence of ethanolamine or choline (Carman 1989; Carman and Henry 1989, 1999; Henry 1982; Paltauf et al. 1992). Yet, the Kennedy pathway contributes to the synthesis of PE and PC under this growth condition (Henry and Patton-Vogt 1998; Kim et al. 1999; Patton-Vogt et al. 1997). For example, the PC synthesized via the CDP–DAG pathway is constantly hydrolyzed to free choline and PA (Patton-Vogt et al. 1997; Xie et al. 1998) by the *SPO14*/*PLD1*-encoded (Rose et al. 1995; Waksman et al. 1996) phospholipase D

[4] In mammalian cells, PS is synthesized by an exchange reaction between PE or PC with serine (Vance 1996).

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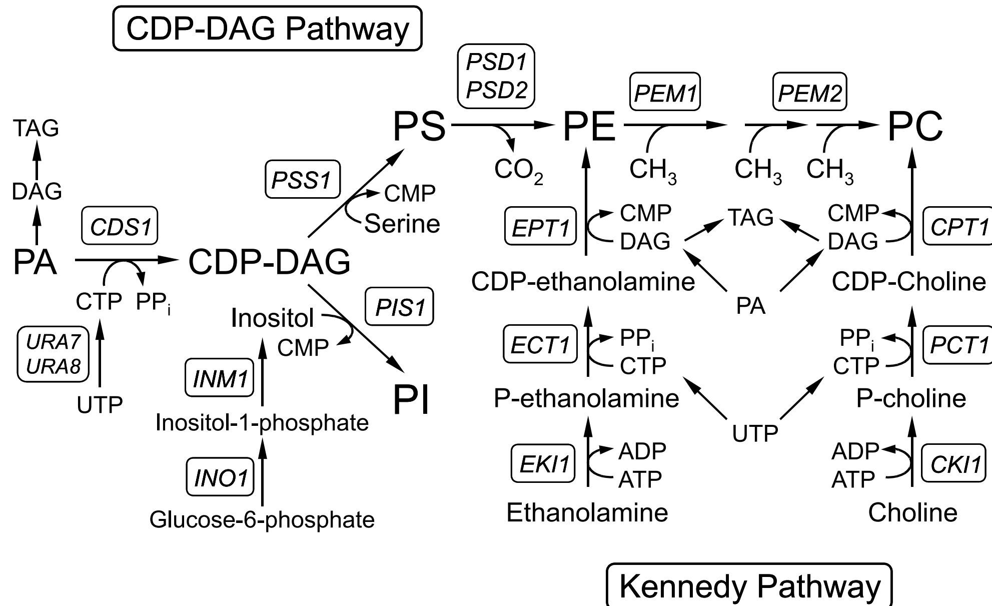
Fig. 1. Pathways for the synthesis of phospholipids in *Saccharomyces cerevisiae*. The pathways shown for the synthesis of phospholipids include the relevant steps discussed in the text. The CDP–DAG and Kennedy pathways are indicated. The known genes that code for enzymes catalyzing individual steps in the pathway are also indicated. PA, phosphatidate; CDP–DAG, CDP–diacylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; P-choline, phosphocholine; P-ethanolamine, phosphoethanolamine; DAG, diacylglycerol; TAG, triacylglycerol.



enzyme. Free choline is incorporated back into PC via the CDP–choline branch of the Kennedy pathway, and PA is incorporated into phospholipids via reactions utilizing CDP–DAG and DG (Carman and Henry 1999; Carman and Zeimetz 1996; Paltauf et al. 1992).

The Kennedy pathway assumes a critical role in phospholipid synthesis when enzymes in the CDP–DAG pathway are defective (Carman and Henry 1989, 1999; Greenberg and Lopes 1996; Paltauf et al. 1992). Mutants in the CDP–DAG pathway require choline for growth and synthesize PC by way of CDP–choline (Atkinson et al. 1980*a*, 1980*b*; Kodaki and Yamashita 1987, 1989; McGraw and Henry 1989; Summers et al. 1988; Trotter et al. 1995; Trotter and Voelker 1995). Mutants defective in the synthesis of PS (Atkinson et al. 1980*a*, 1980*b*) or PE (Trotter et al. 1995; Trotter and Voelker 1995) can also synthesize PC if they are supplemented with ethanolamine. The ethanolamine is used for PE synthesis by way of CDP–ethanolamine. The PE is subsequently methylated to form PC.

Regulation of phospholipid synthesis by inositol

Regulation of phospholipid synthesis by inositol has been extensively characterized and reviewed (Carman and Henry 1989, 1999; Carman and Zeimetz 1996; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al. 1992). A summary of this regulation is presented here. When inositol is supplemented to the growth medium of wild-type cells, the level of PI increases whereas the levels of PS, PE, and PC decrease (Kelley et al. 1988; Klig et al. 1985). These changes are due to genetic and biochemical mechanisms. The expression of genes encoding enzymes responsible for the synthesis of PI and PC is regulated by inositol (Carman and Henry 1989, 1999; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Howe et al. 2002; Paltauf et al. 1992) (Table 1). These genes are maximally expressed when inositol is absent from the growth medium and repressed when inositol is supplemented to the growth medium. Repression by inositol supplementation is enhanced by the inclusion of ethanolamine or choline in the growth medium (Carman and Henry 1989, 1999; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al. 1992). Inositol-mediated regulation involves the regulatory proteins Ino2p, Ino4p, and Opi1p (Carman and Henry 1989, 1999; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al. 1992). Ino2p (Nikoloff et al. 1992) and Ino4p (Hoshizaki et al. 1990) are positive transcriptional regulators whereas Opi1p (White et al. 1991) is a negative transcriptional regulator. Regulation of phospholipid synthesis

Table 1. Phospholipid synthesis genes that are regulated by inositol supplementation.

Gene	Enzyme
INO1	Inositol-1-phosphate synthase
CDS1	CDP–DAG synthase
PSS1/CHO1	PS synthase
PSD1	PS decarboxylase
PEM1/CHO2	PE methyltransferase
PEM2/OPI3	Phospholipid methyltransferase
CKI1	Choline kinase
CPT1	Choline phosphotransferase

Note: The genes listed contain UAS_{INO} element(s) to which the Ino2p–Ino4p complex binds to drive maximal expression of the indicated enzymes when cells are grown in the absence of inositol. The repression of these genes in response to inositol supplementation is mediated by Opi1p.

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by inositol is mediated via a UAS_{INO} *cis*-acting element(s) (Carman and Henry 1989; Kodaki et al. 1991; Lopes et al. 1991; Schuller et al. 1992, 1995) present in the promoters of the structural genes encoding phospholipid synthesis enzymes (Ambroziak and Henry 1994; Carman and Henry 1989, 1999; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Paltauf et al. 1992). The UAS_{INO} element contains a consensus-binding site (5′-CANNTG-3′) for an Ino2p–Ino4p heterodimer, which is required for maximum expression of the coregulated UAS_{INO}-containing genes (Carman and Henry 1999; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Hirsch and Henry 1986; Loewy and Henry 1984; Schwank et al. 1995). Opi1p mediates repression of the coregulated phospholipid synthesis genes through the UAS_{INO} element (Bachhawat et al. 1995) but not by direct interaction (Wagner et al. 1999).

The increased level of PI in response to inositol supplementation is primarily due to a biochemical mechanism (Kelley et al. 1988). Given the low intracellular levels of inositol and the relatively high K_m value for inositol, the synthesis of PI by the PI synthase enzyme is regulated by the availability of inositol (Kelley et al. 1988). Moreover, inositol is an inhibitor of the PS synthase enzyme (Kelley et al. 1988). This regulation also contributes to the decrease in the synthesis of PS and ultimately PE and PC (Kelley et al. 1988).

Regulation of phospholipid synthesis by phosphorylation

Covalent modification by phosphorylation is a major mechanism by which the activity of an enzyme or a regulatory protein is controlled (Calkhoven and Ab 1996; Hung et al. 1997; Kaffman et al. 1998; Karin and Hunter 1995; Komeili and O'Shea 1999; Liu et al. 2000). Enzyme phosphorylation can affect catalytic activity and (or) subcellular localization. Phosphorylation of a regulatory protein can control its localization, stability, or interaction with DNA or other proteins. Data indicate that phospholipid synthesis in yeast is regulated by phosphorylation. Some of this phosphorylation is mediated by protein kinases A and C.

In *S. cerevisiae*, protein kinase A is the principal mediator of signals transmitted through the Ras–cAMP pathway (Broach and Deschenes 1990; Thevelein 1994). Protein kinase A activity is required for proper regulation of growth, progression through the cell cycle, and development in response to various nutrients (Broach and Deschenes 1990; Thevelein 1994). Protein kinase A consists of two catalytic subunits (encoded by *TPK1*, *TPK2*, and *TPK3*) and two regulatory subunits (encoded by *BCY1*). Elevated cAMP levels, which are controlled by adenylate cyclase (encoded by *CYR1*) via the Ras–cAMP pathway, promote dissociation of the regulatory subunits from the catalytic subunits, thus allowing the catalytic subunits to phosphorylate a variety of substrates (Broach and Deschenes 1990; Thevelein 1994). Some of these substrates are enzymes responsible for the synthesis of phospholipids and the transcription factor Opi1p (Table 2).

The activation of the Ras–cAMP pathway in *S. cerevisiae* results in a number of changes in lipid metabolism (Kinney et al. 1990; Quinlan et al. 1992). These changes include an increase in PI synthesis at the expense of PS synthesis and an increase in the synthesis of DAG (Kinney et al. 1990; Quinlan et al. 1992). The decrease in PS synthesis may be attributed to inhibition of PS synthase by protein kinase A mediated phosphorylation (Kinney et al. 1990; Kinney and Carman 1988). The increase in DAG synthesis may be attributed to stimulation of Mg^{2+}-dependent PA phosphatase by protein kinase A mediated phosphorylation (Quinlan et al. 1992). The increase in PI synthesis is not due to the phosphorylation of PI synthase by protein kinase A (Kinney et al. 1990). Instead, PI synthesis increases because of a loss of competition of PI synthase and PS synthase for their common substrate CDP–DAG because of down-regulation of PS synthase by phosphorylation (Kinney et al. 1990). Although the PS synthase reaction in the CDP–DAG pathway is down-regulated by protein kinase A phosphorylation, the overall synthesis of PC is not affected by the activation of the Ras–cAMP pathway (Kinney et al. 1990). The phosphorylation and stimulation of CTP synthetase, choline kinase, and PA phosphatase by protein kinase A is consistent with the increased utilization of the CDP–choline branch of the Kennedy pathway for PC synthesis under this condition. This hypothesis is supported by the fact that phosphorylation site mutants of CTP synthetase (Choi et al. 2003; Park et al. 2003) and choline kinase (Yu et al. 2002) exhibit a decrease in the synthesis of PC by the CDP–choline branch of the Kennedy pathway.

Protein kinase C, which is encoded by the *PKC1* gene, is essential for progression of the *S. cerevisiae* cell cycle (Levin et al. 1990) and plays a role in cell wall formation (Levin and Bartlett-Heubusch 1992). In mammalian cells, protein kinase C plays a central role in the regulation of a host of cellular functions through its activation by growth factors, hormones, and other agonists (Kaibuchi et al. 1985; Persons et al. 1988; Rozengurt et al. 1984). These functions include cell growth and proliferation (Kaibuchi et al. 1985; Persons et al. 1988; Rozengurt et al. 1984). The yeast CTP synthetase and Opi1p proteins are phosphorylated by protein kinase C (Table 2). The analysis of phosphorylation site mutants that are defective in phosphorylation has shown that protein kinase C plays a role in the regulation of phospholipid synthesis.

Phosphorylation of phospholipid biosynthetic enzymes and the transcriptional regulatory protein Opi1p

In this section of the review, we will discuss the phosphorylation of individual enzymes and of the transcriptional regulatory protein Opi1p.

CTP synthetase phosphorylation

The *URA7*-encoded CTP synthetase is phosphorylated on multiple serine residues in vivo (Yang and Carman 1995). In vitro studies have shown that CTP synthetase is a substrate for protein kinase A (Yang and Carman 1996) and protein kinase C (Yang et al. 1996; Yang and Carman 1995). These phosphorylations result in the stimulation of CTP synthetase activity by a mechanism that increases catalytic turnover (Yang et al. 1996; Yang and Carman 1995, 1996). In addition, phosphorylation facilitates nucleotide substrate-dependent tetramerization of the enzyme (Pappas et al. 1998) and

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Table 2. Phosphorylated proteins of yeast (*Saccharomyces cerevisiae*) phospholipid metabolism.

Enzyme	Gene	Protein kinase(s) involved	Phosphorylation site(s)	Effect on function
CTP synthetase	*URA7*	Protein kinase A	Ser^{424}	Stimulation
		Protein kinase C	Ser^{36}, Ser^{354}, Ser^{424}, and Ser^{454}	Stimulation
			Ser^{330}	Inhibition
Choline kinase	*CKI1*	Protein kinase A	Ser^{30} and Ser^{85}	Stimulation
45-kDa Mg^{2+}-dependent PA phosphatase	Unknown	Protein kinase A	Unknown	Stimulation
PS synthase	*CHO1/PSS1*	Protein kinase A	Unknown	Inhibition
Opi1p transcription factor	*OPI1*	Protein kinase A	Ser^{31} and Ser^{251}	Stimulation
		Protein kinase C	Ser^{26}	Inhibition

causes a decrease in the sensitivity of the enzyme to inhibition by CTP (Yang et al. 1996; Yang and Carman 1996). Tetramerization and product inhibition are major mechanisms by which CTP synthetase is regulated (Ostrander et al. 1998; Pappas et al. 1998; Yang et al. 1994).

A combination of biochemical and molecular methods has been used to identify phosphorylation sites in CTP synthetase (Park et al. 1999, 2003). Ser^{424} has been identified as a target site for protein kinase A (Park et al. 1999) and protein kinase C (Choi et al. 2003). Ser^{36}, Ser^{330}, Ser^{354}, and Ser^{454} are also target sites for protein kinase C (Park et al. 2003). Analysis of serine to alanine mutations has shown that phosphorylation at one site affects the phosphorylation of the enzyme at another site (Choi et al. 2003; Park et al. 2003). For example, phosphorylation of Ser^{424} by protein kinase A or protein kinase C is required for maximum phosphorylation and stimulation by protein kinase C (Choi et al. 2003). Moreover, a mutant analysis has shown that the phosphorylation of CTP synthetase at different sites has opposite effects on enzyme activity. The phosphorylation of CTP synthetase at Ser^{36}, Ser^{354}, Ser^{424}, and Ser^{454} results in the stimulation of CTP synthetase activity whereas phosphorylation at Ser^{330} results in the inhibition of activity (Choi et al. 2003; Park et al. 1999, 2003). This regulation is physiologically relevant with respect to phospholipid synthesis. The phosphorylation of these sites correlates with the increase (Ser^{36}, Ser^{354}, Ser^{424}, and Ser^{454}) or decrease (Ser^{330}) in PC synthesis via the CDP–choline branch of the Kennedy pathway (Choi et al. 2003; Park et al. 2003). The mechanism for this regulation has been attributed to the availability of CTP for phosphocholine cytidylyltransferase (Choi et al. 2003; Park et al. 2003), the rate-limiting enzyme in the CDP–choline branch of the Kennedy pathway (Kent and Carman 1999; McMaster and Bell 1994; Vance 1991).

Choline kinase phosphorylation

Choline kinase is phosphorylated on multiple serine residues in vivo, and some of this phosphorylation is mediated by protein kinase A (Kim and Carman 1999). In vitro, protein kinase A phosphorylates choline kinase on a serine residue, and this phosphorylation results in a stimulation of choline kinase activity by a mechanism that increases catalytic turnover (Kim and Carman 1999). The results of biochemical and mutagenesis experiments have shown that protein kinase A phosphorylates choline kinase at Ser^{30} and Ser^{85}, with the former being the major site of phosphorylation (Yu et al. 2002). Experiments using S30A and S85A mutants have revealed that the phosphorylation of Ser^{30} (alone and in combination with Ser^{85}) is responsible for the major regulation of choline kinase activity in vivo. Moreover, the phosphorylation of choline kinase at these residues regulates the synthesis of PC via the CDP–choline branch of the Kennedy pathway (Yu et al. 2002).

PA phosphatase phosphorylation

Two membrane-associated forms (45 and 104 kDa) of the Mg^{2+}-dependent PA phosphatase have been isolated from *S. cerevisiae* (Carman 1997; Lin and Carman 1989; Morlock et al. 1991). These enzymes are regulated differentially by phosphorylation (Quinlan et al. 1992). The 45-kDa Mg^{2+}-dependent PA phosphatase (Morlock et al. 1991) is phosphorylated by protein kinase A whereas the 104-kDa enzyme (Lin and Carman 1989; Morlock et al. 1991) is not phosphorylated (Quinlan et al. 1992). The 45-kDa enzyme is phosphorylated on a serine residue, which results in a stimulation in Mg^{2+}-dependent PA phosphatase activity. The site(s) of phosphorylation has not been determined. Studies with *bcy1* and *cyr1* mutants defective in protein kinase A activity indicate that this phosphorylation occurs in vivo (Quinlan et al. 1992). Moreover, activation of the Ras–cAMP pathway results in an increase in the synthesis of DAG and triacylglycerol (Quinlan et al. 1992), which is consistent with the stimulation of 45-kDa Mg^{2+}-dependent PA phosphatase by protein kinase A phosphorylation (Carman 1997).

PS synthase phosphorylation

PS synthase is phosphorylated by protein kinase A on a serine residue (Kinney and Carman 1988). This phosphorylation results in a reduction in PS synthase activity. Studies with *bcy1* and *cyr1* mutants of the Ras–cAMP pathway have shown that the phosphorylation of PS synthase is relevant in vivo (Kinney and Carman 1988). Moreover, in vivo experiments have shown that under conditions where PS synthase is phosphorylated by protein kinase A, the synthesis of PI increases at the expense of PS and its derivatives PE and PC (Kinney et al. 1990). The target site(s) of phosphorylation has not yet been identified.

Opi1p phosphorylation

In vivo labeling experiments have shown that Opi1p is a phosphoprotein (Sreenivas et al. 2001; Sreenivas and Carman 2003). In vitro studies using a maltose-binding pro-

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tein (MBP) – Opi1p fusion protein have shown that Opi1p is a substrate for protein kinase A (Sreenivas and Carman 2003) and protein kinase C (Sreenivas et al. 2001). Ser^{31} and Ser^{251} are major phosphorylation sites for protein kinase A (Sreenivas and Carman 2003), and Ser^{26} is a major protein kinase C phosphorylation site (Sreenivas et al. 2001). The physiological consequence of the phosphorylation of Opi1p at Ser^{26}, Ser^{31}, and Ser^{251} has been examined by measuring the effects of S26A, S31A, and S251A mutations on the expression of the *INO1* gene, which contains the UAS_{INO} element. The β-galactosidase activity driven by an *INO1-CYC-lacZ* reporter gene in *opi1*Δ mutant cells expressing the S31A and S251A mutant Opi1p proteins is elevated in the absence and presence of inositol when compared with cells expressing wild-type Opi1p (Sreenivas and Carman 2003). The S26A mutation has the opposite effect on *INO1* expression (Sreenivas et al. 2001). These data support the conclusion that phosphorylation of Opi1p at Ser^{31} and Ser^{251} mediates the stimulation of the negative regulatory function of Opi1p whereas phosphorylation at Ser^{26} attenuates Opi1p function (Sreenivas et al. 2001; Sreenivas and Carman 2003). Thus, signals transmitted through the Ras–cAMP and the protein kinase C signaling pathways appear to regulate expression of *INO1* by opposing mechanisms. Additional studies will be required to determine whether the phosphorylation of Opi1p plays a role in the regulation of other UAS_{INO}-containing genes.

The precise mechanism by which phosphorylation via protein kinases A and C mediate Opi1p regulatory activity is not yet known. Although Opi1p mediates its negative regulatory activity through the UAS_{INO} element (Bachhawat et al. 1995), the target of Opi1p has been a mystery because this does not occur by direct interaction (Graves and Henry 2000; Wagner et al. 1999). The recent work of Wagner et al. (2001) has shown that Opi1p interacts with the pleiotropic repressor Sin3p and with the phospholipid synthesis positive transcription factor Ino2p. The availability of the phosphorylation site mutants will permit further studies on the role of phosphorylation by protein kinases A and C on Opi1p interactions with Sin3p and Ino2p and understanding its repressor function in regulating phospholipid synthesis.

Summary and future directions

In the yeast *S. cerevisiae*, phospholipids are synthesized by the CDP–DAG pathway and the Kennedy pathway. These pathways are regulated by genetic and biochemical mechanisms. Expression of several enzymes responsible for the synthesis of PI and PC is regulated by inositol supplementation. Key enzymes that catalyze reactions in the phospholipid biosynthetic pathways have been identified as targets of phosphorylation. Protein kinase A phosphorylates the enzymes CTP synthetase, choline kinase, Mg^{2+}-dependent PA phosphatase, and PS synthase and the transcription factor Opi1p. CTP synthetase and Opi1p are also phosphorylated by protein kinase C. The phosphorylation of these proteins plays a role in regulating their activities and (or) function in phospholipid synthesis.

While a great deal is known about genetic regulation by inositol, we have only begun to understand the role of phosphorylation in regulating phospholipid synthesis in *S. cerevisiae*. A computer analysis of the yeast database predicts that additional enzymes involved in phospholipid synthesis are targets of phosphorylation, not only by protein kinases A and C but also by other protein kinases (e.g., protein kinase G, casein kinases I and II, glycogen synthase kinase-3, calmodulin kinase II). Identification of phosphorylation sites in phospholipid biosynthetic enzymes and the protein kinases involved will lead to the isolation of phosphorylation site mutants. The analysis of these mutants will further our understanding of how phosphorylation regulates phospholipid synthesis.

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