MICROBIOLOGICAL REVIEWS, Mar. 1996, p. 1–20 Vol. 60, No. 1
0146-0749/96/$04.00+0

Genetic Regulation of Phospholipid Biosynthesis in *Saccharomyces cerevisiae*

MIRIAM L. GREENBERG[1] AND JOHN M. LOPES[2]*

Department of Molecular and Cellular Biochemistry and Program in Molecular Biology, Loyola University of Chicago, Maywood, Illinois 60153,[2] and Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202[1]

INTRODUCTION

The membranes of *Saccharomyces cerevisiae* are composed of a typical mixture of phospholipids which serve a general function in cellular compartmentation but may also control the intracellular responses to external stimuli. For example, *S. cerevisiae* synthesizes several phosphorylated forms of phosphatidylinositol (PI) (10, 131) that have been proposed to play a role in signal transduction pathways in multicellular eucaryotes (32). Consequently, it is critical to our knowledge of the biology of a eucaryotic cell that we understand how the eucaryotic cell coordinates the synthesis and assembly of the mixture of lipids that make up the membrane matrix. This topic is enormously complex because it encompasses several levels of biological control, including genetic regulation, cellular localization, and coordination with other cellular processes. Membrane lipids have also been implicated in such vital cellular processes as proliferation and differentiation in multicellular eucaryotes. For instance, phosphoinositides serve as secondary messengers of hormone- or neurotransmitter-mediated induction of cell proliferation. Membrane phospholipids are also involved in other functions pertaining to lipoprotein metabolism (15, 228), cell surface recognition (2), and aging of erythrocytes (128, 129). In addition, fluctuations in phospholipid synthesis are a manifestation of a number of clinical problems, including Alzheimer's disease (176), human immunodeficiency virus type 1 cytotoxicity (145), cystic fibrosis (138), and poliovirus replication (56). Therefore, the biogenesis of membrane phospholipids is an essential process of all eucaryotic cells, and *S. cerevisiae* has proven to be an excellent organism for the study of phospholipid synthesis.

We will review the body of knowledge on the structural genes that are required for the synthesis of membrane phospholipids in *S. cerevisiae*. In doing so, we will describe the plethora of genetic mutants that facilitated the identification of structural genes. This will serve to illustrate how genetics has been essential in the development of our understanding of membrane synthesis. This review will also describe the various regulatory mechanisms that control expression of the phospho-

* Corresponding author. Mailing address: Department of Molecular and Cellular Biochemistry and Program in Molecular Biology, Loyola University of Chicago, 2160 S. First Ave., Maywood, IL 60153. Phone: (708) 216-8454. Fax: (708) 216-8523.

TABLE 1. *S. cerevisiae* phospholipid biosynthetic genes, proteins, and mutants

Gene	Protein	Mutant	Reference(s)
INO1	IPS	*ino1*	31, 34, 102
CHO1 (*PSS*)	PSS	*cho1* and *pss*	8, 9, 116, 168
CHO2 (*PEM1*)	PEMT	*cho2* and *pem1*	112, 208
OPI3 (*PEM2*)	PMT	*opi3* and *pem2*	50, 112, 227
PIS1	PI synthase	*pis*	38, 39, 169
ITR1 and *ITR2*	Inositol transporters (major and minor, respectively)	*itr1* and *itr2*	122, 167
	GPAT or DHAPAT?	*tpa1*	212
	CDP-DGS?	*cdg1*	104
PSD1 and *PSD2*	PSD (two enzymes)	*psd1* and *psd2*	25, 215–217
CTR1 (*HNM1*)	Choline transporter	*ctr1* and *hnm1*	136, 137, 166, 170
CKI	Choline kinase	*cki*	75
CCT1	Phosphocholine cytidylyltransferase	*cct1*	218
EPT1	Ethanolamine phosphotransferase	*ept1*	69
CPT1	Choline phosphotransferase	*cpt1*	68, 70
LCB1 and *LCB2*	Serine palmitoyltransferase (two subunits)		19, 159
FAS1 and *FAS2*	Fatty acid synthase (β and α subunits, respectively)		23, 156
FAS3 (*ACC1*)	Acetyl-CoA carboxylase	*acc1*	1, 59
INO2 (*SCS1*) and *INO4*	Transcriptional activators; required for derepression in response to inositol deprivation	*ino2* and *ino4*	29, 33, 78, 105, 175
OPI1	Transcriptional repressor; required for inositol-mediated repression	*opi1*	51, 224
UME6 (*CAR80*)	General transcriptional repressor	*ume6* and *car80*	206, 226
SIN3 (*CPE1*/*UME4*/*RPD1*/*SDI1*/*GAM1*)	General transcriptional repressor	*sin3*, *cpe1*, *ume4*, *rpd1*, *sdi1*, and *gam2*	83, 202, 205, 219, 229
IRE1	Potential protein kinase required for *INO1* expression		172
	Unknown	*CSE1* (dominant inositol auxotroph)	77
SCS2 (suppressor of *CSE1*)	Unknown		163
SCT1 (allele-specific multicopy suppressor of *ctr1* mutant)	Unknown		149
SLC1	Potential acyltransferase	*SLC1-1* (suppressor of *lcb1* mutant)	160

lipid biosynthetic genes. Although a number of different regulatory mechanisms will be discussed, we will focus on a response to inositol in the growth media because several details of the molecular mechanism for this response are well defined. The identification of the *cis*- and *trans*-acting regulatory elements that are characteristic of the response to inositol has revealed that there are a number of other biological systems in *S. cerevisiae* that are also sensitive to inositol deprivation. Thus, the response to inositol is a complex process that targets phospholipid biosynthesis and other biochemical pathways. We will also discuss how phospholipid biosynthesis is coordinated with other biological processes (e.g., meiosis and mating-type specification) through a mechanism that involves two global transcriptional repressor proteins. Lastly, we will examine the unusual sensitivity of phospholipid biosynthetic gene expression to defects in the basal transcription machinery. Thus, we present our current understanding of the synthesis and regulation of phospholipid biosynthetic gene expression. The *S. cerevisiae* phospholipid biosynthetic genes are listed in Table 1.

GENETIC REGULATION

Biosynthetic Pathways

Phosphatidic acid and CDP-diacylglycerol synthesis. Phosphatidic acid (PA) is the precursor of the major phospholipids present in cell membranes. While much is known about the biosynthesis of PA-derived phospholipids, regulation of the synthesis of PA itself is not well understood. Two routes to PA synthesis in eucaryotic cells have been described (155). Glycerophosphate acyltransferase (GPAT) catalyzes the acylation of glycerol 3-phosphate (G3P) by acyl coenzyme A (acyl-CoA) to form 1-acyl-glycerophosphate (lyso-PA). Alternatively, DHAP acyltransferase (DHAPAT) catalyzes the acylation of dihydroxyacetone phosphate (DHAP) to produce acyl DHAP. Acyl DHAP reductase catalyzes the reduction of acyl DHAP by NADPH to lyso-PA. Acylation of lyso-PA yields PA. In animals, the acyl DHAP pathway is utilized in the synthesis of ether lipids (57). While ether lipids have not been detected in *S. cerevisiae* (46, 146), both GPAT and DHAPAT activities have been observed and characterized (188, 194, 200). In addition, *S. cerevisiae* has acyl DHAP reductase activity, which suggests that PA can be synthesized by either the G3P or the acyl DHAP pathway in this organism (188).

Several aspects of PA biosynthesis in *S. cerevisiae* are not clear. First, the synthesis of PA from lyso-PA has not been characterized. Second, the relative contribution of the G3P and the DHAP pathways to the synthesis of PA and PA-derived phospholipids remains to be elucidated. Thus, while the acyl DHAP pathway is required for ether lipid synthesis in higher eucaryotes, the role of this pathway in *S. cerevisiae* is not clear. This is largely because the enzymes involved in PA biosynthesis have not been well characterized and none of the genes encoding these enzymes have been identified. It is not clear whether GPAT and DHAPAT activities are due to a single enzyme or two separate enzymes. Schlossman and Bell (194) reported that these enzymes have identical properties

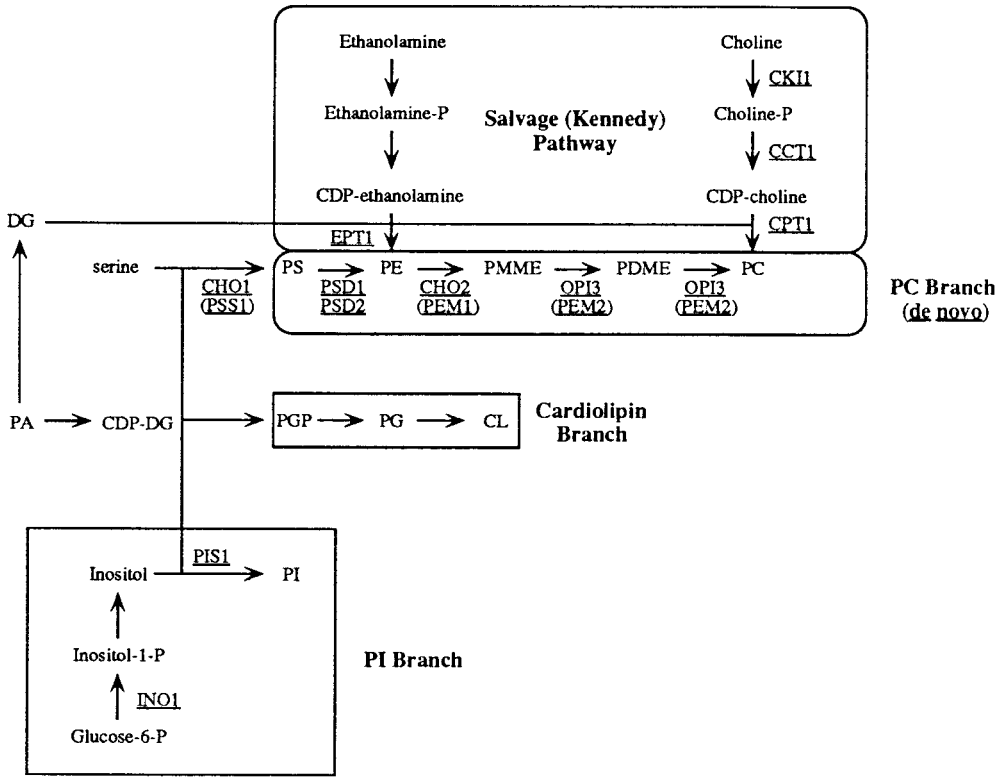

FIG. 1. Schematic depiction of the phospholipid biosynthetic pathways. When the cognate structural gene has been identified, it is designated by underlining (alternate names are given in parentheses). For abbreviations, see the text.

and suggested that the same enzyme may have dual catalytic functions. This is supported by the findings of Tillman and Bell (212), who identified a mutation (*tpa1*) which causes a decrease in both GPAT and DHAPAT activities. Racenis et al. (188), however, showed that these two activities have different pH optima and different degrees of sensitivity to inhibition by *N*-ethylmaleimide. These findings could be consistent with a single enzyme containing two active sites or two separate enzymes. Regulation studies are more consistent with the existence of two different acyltransferases (155, 188). Interestingly, while both GPAT and DHAPAT (and reductase) activities were increased during respiratory growth, the extents of increase in the levels of the two acyltransferases were different. Furthermore, the relative decrease in these two activities differed in the *tpa1* mutant. These data suggest that GPAT and DHAPAT are probably different enzymes. Subcellular fractionation studies indicate that both enzymes are located primarily in the endoplasmic reticulum and not in peroxisomes, the site of the mammalian enzymes (187). The implication of separate enzymes for GPAT and DHAPAT activities in *S. cerevisiae* is that the acyl DHAP pathway may play a significant role in PA biosynthesis in this yeast. As yeast cells do not appear to contain ether lipids, this organism is an excellent model system in which to study the contribution of this pathway to the biogenesis of nonether lipids in eucaryotic cells.

The phospholipid precursor inositol, which is a key regulator of expression of enzymes involved in the synthesis of PA-derived lipids, does not appear to regulate the acyltransferases significantly, although activity of acyl DHAP reductase is slightly reduced (33%) during growth in the presence of exogenous inositol (155). The activities of these enzymes are also not altered by deletion of the mitochondrial genome (155).

CDP-diacylglycerol (CDP-DG) is the liponucleotide intermediate from which diverge the three branches of de novo phospholipid biosynthesis (Fig. 1). These include the PI, phosphatidylcholine (PC), and diphosphatidylglycerol (or cardiolipin [CL]) branches. CDP-DG synthase (CDP-DGS) catalyzes the synthesis of CDP-DG from CTP and PA. The yeast enzyme has been purified to near homogeneity (93). However, the structural gene(s) encoding the enzyme has not been identified. Expression of CDP-DGS is regulated by phospholipid precursors (73). Growth in medium supplemented with exogenous inositol reduced activity to 66% of that measured in unsupplemented medium, and addition of choline or ethanolamine to the inositol-containing medium resulted in further reduction of activity to about 40%. By screening mutagenized cells for inositol excretor mutants, Klig et al. (104) identified a mutant, *cdg1*. The *cdg1* mutant exhibited pleiotropic deficiencies in phospholipid biosynthesis, including reduced CDP-DGS activity, constitutive levels of inositol-1-phosphate synthase (IPS), and elevated amounts of phosphatidylserine (PS) synthase (PSS). Partially purified CDP-DGS enzymes from wild-type and *cdg1* mutant cells were similar with regard to enzymological properties. However, immunoblot analysis of enzyme from cell extracts indicated that the mutant contained less CDP-DGS protein than the wild type did. At least two possibilities are consistent with these data. Two structural genes, one of them *CDG1*, could encode CDP-DGS. In this case, the pleiotropic effects observed in the mutant are secondary consequences of the structural gene defect. Alternatively, *CDG1* may be a regulatory gene, whose product controls expression of several phospholipid biosynthetic enzymes. Clarification of these possibilities awaits cloning of the *CDG1* gene.

Phosphatidylinositol branch. In yeast cells, PI can be synthesized directly from exogenous inositol or de novo from glucose-6-phosphate (225). The two structural genes *INO1* and *PIS1* are required for the de novo synthesis of PI from glucose-6-phosphate. The *INO1* gene encodes the soluble IPS enzyme (31, 102), which catalyzes the conversion of glucose-6-phosphate to inositol-1-phosphate, which is subsequently dephosphorylated to inositol (27, 28). The membrane-associated *PIS1* gene product, PI synthase (PIS), converts inositol and CDP-DG to PI (38, 39). The essential nature of this pathway is best evidenced by the fact that yeast strains which do not synthesize inositol die when grown in media lacking inositol. This process is referred to as inositolless death and probably requires the production of an unknown protein, since inhibitors of protein synthesis will specifically block cell death (60). Furthermore, disruption of the *PIS1* gene has revealed that this gene is essential for viability (162). Consequently, PI is an essential component of yeast membranes. It is therefore not entirely surprising that the biochemical steps required for PI synthesis are regulated at several levels.

From a biochemical perspective, it makes sense that yeast cells growing in the presence of exogenous inositol would repress production of the *INO1* gene product, which is required to synthesize inositol endogenously. Invoking the same logic, one would expect that synthesis of the *PIS1* gene product should not be affected by growth in the presence of inositol, since it is needed to synthesize PI from inositol. These two predictions were initially borne out in studies that examined the effect of inositol on the production of the IPS and PIS enzymes. These studies show that while IPS activity and subunit concentrations are significantly reduced in cells grown in the presence of inositol (27, 34), PIS subunit levels are not affected (39). However, it should be noted that inositol does affect the function of the PIS, because the K_m of this enzyme for inositol is ninefold higher than the intracellular inositol concentration (92). Consequently, while PIS subunit concentrations are not affected by inositol, growth in the presence of exogenously supplied inositol does increase the level of PI in the membrane from 10 to 27% (92). This increase in PI concentration occurs at the expense of phosphatidylserine (PS) synthesis because both the PIS and PSS enzymes compete for the common precursor, CDP-DG, and inositol is a noncompetitive inhibitor of PSS (92).

It is important to note the impact that studies on expression of the *INO1* gene have had on shaping our current understanding of the response to inositol in growth media. As mentioned above, the earliest evidence that inositol supplementation elicited a change in control of phospholipid biosynthesis was the dramatic reduction in IPS activity and subunit levels when cells were grown in media that contained inositol (27, 34). The cloning of the *INO1* gene (102) made it possible to examine if the response to inositol was due to transcriptional regulation. Quantitative Northern (RNA) blot hybridization clearly showed that a 1.8-kb *INO1* transcript was most abundant when cells were grown in either the absence of inositol and choline or the presence of choline by itself (66). The addition of inositol to the growth medium resulted in a 90% reduction in the level of the *INO1* mRNA, whereas inositol and choline added in conjunction resulted in a 97% reduction. Therefore, the reduction in IPS activity correlates with a decrease in the abundance of the *INO1* gene transcript. Furthermore, the *INO1* gene was not transcribed in strains mutated at the *INO2* or the *INO4* positive regulatory genes and was constitutively transcribed in a strain containing a mutant allele of the *OPI1* negative regulatory gene (66). This pattern of transcriptional regulation has now been observed with a number of genes involved in the synthesis of phospholipids.

The *INO1* promoter was also the first to be examined for the *cis*-acting elements required for the inositol response. Deletion analyses of the *INO1* promoter fused to the *lacZ* reporter gene combined with fusions of *INO1* promoter fragments to the *CYC1-lacZ* reporter suggested the existence of a repeated element required for the inositol response (65, 141). These studies and a computer-aided examination of the *INO1* promoter identify nine potential copies of a repeated 10-bp element (designated UAS_{INO}). In addition to the UAS_{INO} element, a general repressor-binding site ($URS1^{INO1}$) is present in the *INO1* promoter (142). The role of the $URS1^{INO1}$ element is to abate *INO1* expression generally both under repressing and derepressing growth conditions. Both the UAS_{INO} and $URS1^{INO1}$ regulatory elements are discussed more thoroughly below.

In the process of examining the *INO1* promoter, the observation was made that the *INO1* gene could be expressed in the absence of the UAS_{INO} elements. That is, deletions that removed the entire promoter region upstream of the TATA box yielded elevated constitutive expression of an *INO1-lacZ* reporter gene (65, 141). The constitutive expression observed with the *INO1* promoter deletion is dependent on the presence of the TATA box, since removal of the TATA box sequences yield a null promoter (141). Particularly interesting was the isolation of a dominant mutant allele of the *INO1* gene (139) called *INO1-100* (209). This mutant allele was isolated as a suppressor of the inositol auxotrophy of an *ino2 ino4* double-mutant strain (i.e., activatorless). Surprisingly, the mutation which allows for expression of the *INO1* gene, in the absence of its cognate activators, is a deletion (sequences −366 to −128) of the UAS_{INO} elements from the *INO1* promoter (209). This is surprising, since removal of UAS elements from promoters usually yields repressed levels of expression. For example, deletion of the UAS_{INO} element from the *CHO1* promoter yielded constitutive repressed levels of *CHO1-lacZ* expression (12). It is now known that a 20-bp deletion (−247 to −228) of the *INO1* promoter, which does not remove any of the UAS_{INO} elements, will also suppress the inositol auxotrophy of the *ino2 ino4* double-mutant strain (209). While it is still not clear how the *INO1* gene can be expressed in the absence of the UAS_{INO} elements, the most likely explanation is that basal transcription dictates *INO1* expression. It is also likely that the 20-bp *INO1* promoter sequence from −247 to −228 includes a regulatory element that coordinates general and specific transcription factors that regulate *INO1* expression in response to inositol.

The gene that encodes the PIS enzyme was cloned by complementation of a mutant (*pis*) that required high levels of inositol for growth (171) because of a higher K_m for inositol than the wild-type enzyme (119, 169). As noted above, the subunit levels of the PIS enzyme are not affected by inositol (39). Consequently, the *PIS1* clone has been used to determine that the *PIS1* transcript is also constitutively expressed (108). While it is not surprising that expression of this gene would not be responsive to inositol (discussed above), it is curious that the promoter of the *PIS1* gene includes a single copy of a potential UAS_{INO} element (162). Thus, either this UAS_{INO} element is nonfunctional or it is silenced in the context of the native *PIS1* promoter. It is not currently possible to distinguish between these possibilities. Another potential *PIS1* promoter *cis*-acting element was identified by studies in which *MCM1* protein-binding sites were isolated from the yeast genome (120). Two *MCM1* sites are found in the *PIS1* promoter. These findings warrant a formal examination of the role of *MCM1* in transcription of the native *PIS1* gene.

Phosphatidylcholine branch: de novo pathway. PC is the major phospholipid present in eucaryotic cell membranes. In *S. cerevisiae*, PC can be synthesized from CDP-DG de novo (179, 225) (Fig. 1). The first reaction in the PC pathway synthesizes PS from CDP-DG and free serine by using PSS. PS is subsequently decarboxylated by PS decarboxylase (PSD) to phosphatidylethanolamine (PE), which is sequentially methylated to form PC. The latter step involves three methylation reactions which are catalyzed by two methyltransferases. All of the enzymes in this branch of the pathway are known to be membrane associated (179, 225, 230). Here, we will review the information regarding the regulation of each of these reactions. For simplification purposes, the discussion will proceed in the order of the enzymatic reactions (CDP-DG→PS→PE→phosphatidyl-monomethyl ethanolamine [PMME]→PC).

The first reaction in the pathway is catalyzed by PSS (Fig. 1), which was defined by mutants that failed to grow in the absence of choline (8, 9, 116, 168), i.e., *cho1* (also *pss*) mutants. The *cho1* mutants are defective in the synthesis of PS (8, 9, 116) and lack PSS activity (168). However, the *cho1* mutants are able to grow in the presence of either ethanolamine or choline owing to the ability of yeast to synthesize PC via a salvage pathway (discussed below). The gene that encodes the PSS activity, *CHO1* (*PSS*), was independently cloned by several groups using similar strategies, namely, complementation of the choline auxotrophy of *cho1* (*pss*) mutant strains (99, 132, 165). The cloned gene was used to generate null mutants which had the same phenotype (i.e., choline auxotrophy) as the original *cho1* and *pss* mutants (13, 62).

Since expression of the *INO1* gene had previously been shown to be regulated at the level of transcript abundance (66), the cloned *CHO1* gene was used to determine if expression of the *CHO1* gene was regulated similarly. Northern blot and quantitative slot blot hybridization analyses revealed that a 1.2-kb *CHO1* transcript was also regulated at the level of transcript abundance (13). Expression of the *CHO1* gene is maximal in the absence of inositol and choline and is repressed approximately five- to sixfold by the addition of inositol and choline to the growth media. Moreover, derepressed expression of the *CHO1* gene was found to be sensitive to mutations in the *INO2* and *INO4* activator genes and the *OPI1* repressor gene (13). These experiments established *CHO1* as the first gene known to be coregulated with *INO1* in response to inositol and choline supplementation. This observation led to an investigation of the *CHO1* promoter in search of a UAS_{INO} element common to those observed in the *INO1* promoter. The *CHO1* promoter (and amino terminus) was fused to the *lacZ* reporter gene and dissected in two independent studies involving promoter deletion analyses (12, 110). These promoter analyses identified a single region in the *CHO1* promoter that shared homology to the UAS_{INO} element found in the *INO1* promoter and was required for the response to inositol. Curiously, while the single UAS_{INO} element in the *CHO1* promoter accounts for the response to inositol, most of the *CHO1* expression must be dictated by another promoter element (12, 110). The precise location of this other potential UAS element has yet to be determined, but one candidate is an ABF1-binding site that was identified by sequence inspection (198). One of the features of ABF1-binding sites is that they provide weak UAS activity in vivo but act synergistically with other weak UAS elements to give strong UAS activity (17). Consequently, the ABF1-binding site may enhance the activity of the UAS_{INO} element in the *CHO1* promoter, but this possibility will have to be tested formally.

The second reaction in the pathway is catalyzed by PSD, which decarboxylates PS to PE (Fig. 1). One of the genes that encodes the PSD activity (*PSD1*) was cloned by two research groups, using different strategies. One strategy was complementation of an *Escherichia coli* temperature-sensitive *psd* mutant (25). The other strategy was hybridization with a degenerate oligonucleotide probe to a region of the enzyme that is conserved between *E. coli* and *CHO* (Chinese hamster ovary) cells (215). However, strains containing *psd1-Δ1::TRP1* null mutant alleles still retained approximately 4 to 12% of the PSD activity (215) and did not have detectable growth requirements (25, 215). These observations suggested the existence of a second PSD-encoding gene (25, 215). The second PSD-encoding gene (*PSD2*) was recently cloned by complementation of the ethanolamine auxotrophy of a *psd1-Δ1::TRP1 psd2* double-mutant strain (216, 217). The identification of the two *PSD* genes has permitted generating null alleles of each gene. Strains containing null alleles of either *PSD* gene do not have detectable growth phenotypes (25, 215, 216). However, a strain containing null alleles of both *PSD* genes requires ethanolamine for growth, completely lacks PSD enzyme activity, and converts very little [^{3}H]serine-labeled PS into PE in vivo (217). The existence of mutant alleles of the two *PSD* genes has permitted localization of the PSD enzyme activities. The *PSD1*-encoded enzyme represents approximately 90% of the PSD activity (25, 215) and is localized in the mitochondrial membrane (230). The *PSD2*-encoded enzyme accounts for the remaining PSD activity (216) and localizes to a membrane fraction with fractionation properties similar to both vacuoles and the Golgi apparatus (217).

There are two separate studies that examined regulation of PSD activity in response to inositol and choline supplementation. One study shows that PSD activity is responsive to inositol and choline (125), whereas the other study shows that choline alone elicits a response (21). However, these results may not be in conflict, since it is not known if the medium used in the latter study (21) contained inositol. In the former study (125), the enzyme activity was most highly elevated when cells were grown in the absence of inositol and choline and was reduced approximately fourfold upon inositol and choline supplementation. Moreover, the response to inositol was dependent on wild-type alleles of the *INO2*, *INO4*, and *OPI1* regulatory genes, demonstrating that PSD activity is coregulated with the other activities in the pathway. Consistent with the response to inositol and choline, the sequence of the *PSD1* promoter reveals two potential UAS_{INO} elements (25) although the *PSD2* promoter region does not appear to contain a UAS_{INO} element (216).

The final three steps in the synthesis of PC de novo involve the sequential methylation of PE (179, 225) (Fig. 1). These three reactions are catalyzed by two methyltransferases, the phosphatidylethanolamine methyltransferase (PEMT) and the phospholipid methyltransferase (PMT). PEMT catalyzes primarily the first methylation reaction, and, consequently, PEMT mutant strains (*cho2* and *pem1*) accumulate elevated levels of PE (112, 208). PMT catalyzes the last two methylation reactions, and PMT mutants (*opi3* and *pem2*) accumulate elevated levels of PMME and phosphatidyl-dimethyl ethanolamine (PDME) (50, 112, 227). There is some controversy regarding the phenotype of the strains lacking the PEMT and PMT activities. Yamashita et al. reported that such mutant strains (*pem1* and *pem2*) were choline auxotrophs (227). Conversely, Henry's group found their mutant strains (*cho2* and *opi3*) to be choline prototrophs (150, 208). Both groups also generated null alleles of the two genes, which failed to resolve the controversy (112, 150, 208). That is, the null mutants created by Yamashita's group were choline auxotrophs (112) whereas the null mutants constructed by Henry's group were choline pro-

totrophs (150, 208). One explanation for this apparent discrepancy may be that the genetic background for the strain used by Yamashita et al. harbored a second mutation that, in combination with *pem1* or *pem2* mutant alleles, created a choline growth requirement. In support of this possibility, strains harboring *cho2 opi3* (150) or *cho2 cdg1* (208) mutant combinations require choline for growth. However, it is difficult to determine if this observation resolves the controversy because genetic segregation studies have not been reported for the *pem1* and *pem2* mutant alleles.

The *CHO2* (*PEM1*) and *OPI3* (*PEM2*) genes were cloned by complementation and sequenced (111, 150, 208). It had already been demonstrated that the PEMT and PMT activities were maximal when the strains were grown in unsupplemented media and were reduced 72% (PEMT) and 63% (PMT) when the strains were grown in media supplemented with inositol and choline. Northern blot analyses revealed that these differences in enzyme activity are caused by regulation of transcript abundance (42, 108). The *PEM1* (*CHO2*) and *PEM2* (*OPI3*) gene promoter sequences downstream of −336 (*PEM1*) and −177 (*PEM2*) are required for inositol-specific regulated expression of a *CYC1-lacZ* reporter gene (108). Both of these regions contain several sequence elements that resemble the UAS_{INO} element (108). Consequently, the *CHO2* (*PEM1*) and *OPI3* (*PEM2*) genes are clearly members of the inositol-specific regulon. Studies of the *CHO2* (*PEM1*) promoter also show that the various UAS_{INO} elements confer vastly different levels of expression to the *CYC1-lacZ* reporter gene. However, none of the UAS_{INO} elements by themselves are sufficient to account for the full level of *CHO2* (*PEM1*) gene expression. The data also show that the ability of the various *CHO2* (*PEM1*) UAS_{INO} elements to stimulate transcription of the *CYC1-lacZ* reporter gene is additive (108), which suggests that expression of the *CHO2* (*PEM1*) gene may require the combined effect of several UAS_{INO} elements. In the case of the *PEM2* (*OPI3*) gene, an additional regulatory element (GRF1 site) was discovered adjacent to the UAS_{INO} element (108). Studies with synthetic oligonucleotides revealed that the *PEM2* UAS_{INO} element is required for inositol-specific regulation and the putative GRF1 site is required for high constitutive expression of the *CYC1-lacZ* reporter gene. When combined, these two elements provide elevated and regulated expression of the reporter gene (108). This organization is reminiscent of the *CHO1* (*PSS1*) promoter (discussed above), in which the UAS_{INO} element provides the inositol-specific regulation but an additional regulatory element (possibly ABF1) is required for full expression (110).

Phosphatidylcholine branch: salvage pathway. In addition to the de novo PC biosynthetic pathway, *S. cerevisiae* can utilize a salvage pathway for PC biosynthesis (Fig. 1). The salvage pathway was originally described by Kennedy and Weiss (95) and utilizes ethanolamine and choline to synthesize PC. The first step in this pathway involves the rapid phosphorylation of ethanolamine and choline by choline kinase. At present, there is some debate about whether there are separate enzymes for phosphorylation of these two substrates. However, it has been shown that the enzyme encoded by the *CKI* gene of *S. cerevisiae* phosphorylates both substrates when expressed in either *S. cerevisiae* or *E. coli* (75). The second step in the pathway is the conversion of phosphoethanolamine and phosphocholine to CDP-ethanolamine and CDP-choline, respectively. The conversion of phosphocholine to CDP-choline is catalyzed by phosphocholine cytidylyltransferase, which is encoded by the *CCT1* gene (218). The final step involves CDP-ethanolamine and CDP-choline condensation with DG to form PE and PC, respectively. The enzymes that carry out these final reactions are encoded by the *EPT1* and *CPT1* genes, respectively (68–70).

The regulation of transcription of several of the genes encoding PC salvage pathway enzymes has been examined. Three lines of evidence suggest that expression of the *CKI* gene is regulated in response to inositol supplementation. Northern blot hybridization indicates that the abundance of a 1.9-kb transcript is regulated in response to inositol and choline supplementation (76). Moreover, expression of a *CKI* promoter-*lacZ* fusion gene is regulated in a pattern consistent with that of the native transcript (76), as is the activity of the native enzyme (76, 151). Consistent with this pattern of regulation, the promoter of the *CKI* gene includes a copy of the UAS_{INO} element.

Quantitative reverse transcriptase-PCR studies have shown that *CPT1* transcript abundance is also reduced 69% in response to inositol and choline supplementation (151). This level of regulation is consistent with the observed 68% reduction in enzyme activity under the same growth conditions, as reported by McMaster and Bell (151) but in conflict with the 22% reduction reported by Hosaka et al. (76). Nevertheless, the promoter of the *CPT1* gene includes a potential UAS_{INO} element, which suggests that *CPT1* is a member of the inositol-specific regulon. However, it has yet to be determined if the UAS_{INO} elements present in the promoters of the *CPT1* and *CKI* genes are functional and responsible for the inositol-specific response. In addition, it will also be interesting to determine if expression of the *CPT1* and *CKI* genes is dependent on the *INO2* and *INO4* activator genes.

Unlike the *CKI* and *CPT1* genes, expression of the *CCT1* gene is not dramatically affected by inositol and choline. Quantitative reverse transcriptase-PCR revealed that inositol alone does not affect *CCT1* transcript abundance whereas choline alone or in combination with inositol yields modest reductions (58 and 32%, respectively) in transcript abundance (151). This pattern of expression is consistent with the activity of this enzyme under the same growth conditions (151). Consequently, it does not appear that *CCT1* expression is coordinated with that of the other phospholipid biosynthetic genes. However, the enzyme activity encoded by the *CCT1* gene has been shown to be rate limiting when cells are grown at choline concentrations below 100 μM. This suggests that under specific growth conditions, such as limiting choline, regulation of the CCT activity may play a critical role in the synthesis of PC through the salvage pathway.

Inositol and choline transporters. As discussed above, synthesis of PI can occur by two different mechanisms. PI can be synthesized de novo from glucose or from exogenous inositol. Likewise, PC can be synthesized de novo or by a salvage pathway that requires exogenous choline. For yeast cells to use exogenous inositol and choline in PI and PC synthesis, these precursors must be transported into the cell. For this purpose, yeast cells have membrane-associated inositol and choline transporters. There are two inositol-specific transporters, encoded by the *ITR1* and *ITR2* genes (167). Both genes were cloned by their ability to complement a UV-generated mutation (164) in the *ITR1* gene. The mutation in the *ITR1* gene is sufficient to yield a mutant phenotype, because the *ITR1* gene is transcribed at substantially higher levels than the *ITR2* gene (122, 167). Consistent with this observation, a null allele of the *ITR2* gene does not have a detectable mutant phenotype and the ability of the *ITR2* gene to complement the *itr1* mutant allele is multicopy dependent. That is, the *ITR1* gene will complement the *itr1* mutant phenotype in single copy but the *ITR2* gene will do so only in multicopy.

The first indication that the *ITR1* gene was part of the

inositol-responsive regulon was suggested by a study that reported a reduction of enzymatic activity when cells were grown in the presence of inositol (164). More recently, this reduction in activity has been correlated with a reduction in the steady-state level of the *ITR1* transcript (122). Since this is the more highly transcribed of the two inositol transporter genes, it may be sufficient to account for the reduction in inositol transport activity. The *ITR1* promoter includes a potential UAS_{INO} element (122). Transcription of the *ITR1* gene is sensitive to mutations in the regulatory cascade that controls expression of the phospholipid biosynthetic genes (i.e., *ino2*, *ino4*, and *opi1*) (122). It is not known if the *ITR2* gene is subject to the same transcriptional control.

The inositol transport system is also subject to another novel form of regulation, which involves irreversible Itr1 enzyme inactivation (122). Recently, it was shown that the inactivation of the Itr1 enzyme accompanies endocytic internalization followed by degradation in the vacuole (121). This conclusion is based on experimental evidence which shows that Itr1 enzyme degradation is dependent on the *END3*/*END4* endocytic pathway and on the *PEP4*-encoded vacuolar protease (121). This is the first evidence of enzyme inactivation and protein degradation as mechanisms for regulation of phospholipid biosynthesis.

The cloning of the two *ITR* genes permitted construction of null mutant strains (122). Studies with *ITR* deletion strains clearly show that regulation of phospholipid biosynthetic gene expression requires inositol transport, since an *itr1 itr2* double-mutant strain is defective in repression of the *INO1* gene (122). This suggests that the initial step of the inositol response is the uptake of inositol.

Another enzyme that is coordinately regulated with the phospholipid biosynthetic enzymes is the choline transporter (79), which is encoded by the *CTR1*/*HNM1* gene (136, 161). The *CTR1* gene was defined independently by several different mutant phenotypes. For example, two different mutant selection schemes took advantage of the property that growth of yeast cells is sensitive to chemicals such as nitrogen mustard (137) and hydroxyethylhydrazine (170). Both of these chemicals are transported into the yeast cell via the choline transporter. Hydroxyethylhydrazine is an inhibitor of all three methylation reactions required to synthesize PC de novo. Thus, yeast cells will grow in the presence of hydroxyethylhydrazine only if choline is present, because PC can be synthesized by the Kennedy (salvage) pathway. A defect in choline transport (*ctr1*) eliminated the ability of choline to rescue cells from hydroxyethylhydrazine. In the case of nitrogen mustard, a recessive mutant (*hnm1*) that was hyperresistant to this chemical was obtained. The phenotype is due to a defective choline transporter that is unable to transport nitrogen mustard. The *hnm1* mutant was found to be allelic to the *ctr1* mutant (136). More recently, the *SCT1* gene was cloned as an allele-specific multicopy suppressor of a *ctr1* defect (149). The function of the *SCT1* gene product is currently not known.

The *CTR1* gene has been cloned (166), and its regulation has been examined in two independent studies (136, 161). The data show that expression of both the native *CTR1* gene and a *CTR1-lacZ* reporter gene is regulated in response to inositol and choline. Deletion analysis of the *CTR1* promoter, fused to *lacZ*, identified two regions that are required for *CTR1-lacZ* expression (136). One region (−352 to −265) includes a UAS_{INO} element and is required for the inositol response. Regulation of *CTR1-lacZ* gene expression from this UAS_{INO} element requires the *INO2* and *INO4* activator genes and is sensitive to mutations in the *OPI1* repressor gene. These observations indicate that *CTR1* is a member of the inositol-responsive regulon. Another region of the *CTR1* promoter that contributes to expression of the *CTR1-lacZ* gene was also identified. Curiously, expression from this region (−152 to −1) was dependent on the *INO4* gene but not on the *INO2* gene (136). This is the first example of a promoter element that requires one of these transcriptional regulators but not both. The implications of this observation are discussed below. Since the normal regulatory pattern of phospholipid biosynthetic gene expression includes enhanced repression when both inositol and choline are present, it would be valuable to determine if this level of repression can still be achieved in a *ctr1* mutant strain.

Cardiolipin branch. The phospholipid CL, or diphosphatidylglycerol, is ubiquitous in eucaryotes. It is unique in structure, subcellular localization, and potential function. Because it is found predominantly in the mitochondrial inner membrane (26, 30, 86), it is an excellent marker for mitochondrial biogenesis. CL is required for activity of several mitochondrial enzymes (90, 190, 213, 214), and some experiments suggest that it may be required for protein import (35, 36). Synthesis of CL from G3P and CDP-DG proceeds in three steps, catalyzed by phosphatidylglycerol phosphate (PGP) synthase, PGP phosphatase, and CL synthase (Fig. 1). The first two reactions are similar in procaryotes and eucaryotes. However, the CL synthase reaction in eucaryotes differs from the bacterial reaction. In *S. cerevisiae*, CDP-DG is a substrate in the synthesis of CL, while in procaryotes, two molecules of phosphatidylglycerol condense to form CL but CDP-DG is not a substrate (26, 67, 157, 201, 210). Exhaustive genetic and molecular screens to identify the yeast genes encoding the CL biosynthetic enzymes have been unsuccessful. To date, the structural genes encoding these enzymes have not been cloned in any eucaryotic organism.

Regulation of CL synthesis, as measured by activity of CL biosynthetic enzymes, indicates that two sets of factors affect CL synthesis: cross-pathway control and factors affecting mitochondrial development. Regulation by cross-pathway control is genetically unique compared with regulation of the PI and PC branches of phospholipid synthesis. Expression of PGP synthase (but not PGP phosphatase and CL synthase) is decreased by addition of exogenous inositol to growth medium. This decrease is evident only in cells that can synthesize PC. However, unlike enzymes in general phospholipid synthesis, PGP synthase is not subject to regulation by the *INO2-INO4-OPI1* regulatory genes (49, 94, 210).

An early study by Jakovcic et al. (86) showed that relative CL content in yeast mitochondrial membranes is increased during growth under conditions which favor mitochondrial development (i.e., nonfermentable carbon source, aerobic growth, rho^+ cells). More recent investigations indicate that these factors affect expression of PGP synthase (44) but not PGP phosphatase and CL synthase (94, 210). Regulation of PGP synthase by these factors is independent of cross-pathway control. PGP synthase expression is never fully repressed, even during growth in the absence of oxygen, indicating that this enzyme may be essential. Thus, the amount of PGP synthase relative to mitochondrial protein appears to be the same in the presence and absence of oxygen. Since mitochondrial volume is greater in the presence of oxygen, the total amount of enzyme must be regulated accordingly.

In summary, regulation of CL synthesis by both sets of factors is exerted at the committed step in the pathway, PGP synthase. Apparently, regulation by cross-pathway control is mediated by a different regulatory circuit from that which controls general phospholipid synthesis. Cloning of the structural genes for all three of the biosynthetic enzymes and identifica-

tion of regulatory genes will be necessary to determine the molecular basis of regulation and to understand the function of CL and the intermediates PGP and phosphatidylglycerol.

Sphingolipids. Two sphingolipid biosynthetic genes have been cloned and sequenced (19, 159). The *LCB1* and *LCB2* genes are believed to encode subunits of serine palmitoyltransferase, the first and committed step in the synthesis of the ceramide moiety of sphingolipids. Computer-aided examination of the promoters of these two genes did not identify any UAS_{INO}-like elements. Thus, it seems unlikely that expression of these two genes is coregulated with phospholipid biosynthetic gene expression, although this possibility has not been formally ruled out. The *lcb1* mutant strains require long-chain fatty acids for growth (19). This growth requirement can also be alleviated by an extragenic suppressor mutant, *SLC1-1* (160). The *SLC1* gene was cloned and encodes a protein product that is homologous in sequence and activity to the 1-acyl-*sn*-glycerol-3-phosphate acyltransferase protein of *E. coli* (160). An examination of the promoter for the *SLC1* gene identified two potential UAS_{INO} elements, although it is not known if *SLC1* gene expression is inositol responsive.

Interestingly, the activity of another sphingolipid biosynthetic enzyme, phosphatidylinositol:ceramide phosphoinositol transferase (IPC synthase), is affected by growth in the presence of inositol (107). In contrast to regulation of phospholipid biosynthetic gene expression, the activity of the IPC synthase is increased twofold when cells are grown in the presence of inositol. This would suggest that IPC synthase is not a member of the phospholipid biosynthetic gene regulon. However, induction of IPC synthase in response to inositol is dependent on the *INO4* regulatory gene but not the *INO2* gene. This situation is reminiscent of the one described above for the *CTR1* gene. The implications of these observations are discussed below.

Fatty acids. Saturated fatty acid synthesis in *S. cerevisiae* requires three proteins. Acetyl-CoA carboxylase, encoded by the *ACC1* gene (also called *FAS3*), catalyzes the initial rate-limiting step, synthesis of malonyl-CoA (1, 59). Malonyl-CoA serves as a substrate for the fatty acid synthase complex which is composed of two proteins with an $(\alpha\beta)_6$ stoichiometry. The α and β subunits are encoded by the *FAS2* and *FAS1* genes, respectively (23, 156). It has been shown that expression of the *FAS1*, *FAS2*, and *ACC1* (*FAS3*) genes is regulated in response to fatty acid and inositol supplementation (22, 24, 195). The responses to fatty acids and inositol appear to be dictated by different sets of *cis*- and *trans*-acting regulatory elements. That is, the response to inositol has been shown to be dictated by copies of the UAS_{INO} element (also called UAS_{FAS} or ICRE [inositol/choline responsive element]) present in the promoters for these three genes (22, 24, 195) and requires the products of the *INO2* and *INO4* activator genes (24, 196). However, the response to fatty acids requires sequences outside of the UAS_{INO} elements. The responses to fatty acid and inositol supplementation are cumulative, such that the addition of both precursors is more repressive than the addition of either precursor individually. These observations demonstrate that expression of the fatty acid biosynthetic genes is coordinated with that of the phospholipid biosynthetic genes (response to inositol) but that the genes are also coordinately regulated in a distinct manner (response to fatty acids). The *cis*- and *trans*-acting elements required for the fatty acid response have yet to be identified.

It is important to note that in some cases the response to inositol is modest. For example, expression of *FAS1-lacZ* and *FAS2-lacZ* reporter genes in the presence of inositol is reduced a mere 20% (22, 195). Consequently, other factors must have a more pronounced effect on regulation of expression of these genes. The ability to respond to two different signals (fatty acids and inositol) independently provides the cell with a sensitive mechanism for control of fatty acid synthesis. However, most of the expression of the fatty acid biosynthetic genes is dictated by control elements that ensure a high level of expression but are not part of the regulated responses. An examination of the promoters for the *FAS1*, *FAS2*, and *ACC1* genes reveals several potential binding sites for Gcr1, Abf1, Grf1 (Rap1), and Reb1. Removal of potential Gcr1, Grf1 (Rap1), and Abf1 sites from the *FAS1* promoter results in a 40% reduction in *FAS1-lacZ* expression (22). Likewise, removal of sequences from the *FAS2* promoter that include a potential Gcr1-binding site results in a dramatic 90% reduction in *FAS2-lacZ* expression (22). Similarly, deletion of sequences from the *FAS3* (*ACC1*) promoter that contain putative Abf1-, Reb1-, and two Grf1-binding sites reduces expression of a *FAS3-lacZ* fusion gene by 54% (24). In all these cases, the deletions severally reduce expression without affecting the response to either fatty acid or inositol supplementation.

While the expression of the fatty acid biosynthetic genes is modestly responsive to inositol, the regulatory pattern is consistent with that of the phospholipid biosynthetic genes. That is, repression of gene expression in response to inositol supplementation is dependent on copies of the UAS_{INO} elements. The promoters of the *ACC1* and *FAS1* genes each include two copies of UAS_{INO} elements, although deletion analyses suggest that only one copy is functional in either case (22, 24). In the promoter for the *FAS2* gene, there is only one copy of a UAS_{INO} element, and it has been shown to be functional (22). Furthermore, for all three genes, it has been demonstrated that derepression of gene expression, in response to inositol deprivation, requires the *INO2* and *INO4* activator genes. Therefore, the fatty acid biosynthetic genes are clearly members of the phospholipid biosynthetic gene regulon.

Cross-Pathway Regulation

The relative phospholipid composition in eucaryotic membranes is not random. On the contrary, the major membrane phospholipids PI, PS, PC, and PE (and CL in the mitochondrial membrane) are present in defined concentrations which are similar in *S. cerevisiae* and higher eucaryotes. Even so, cells are capable of responding to environmental changes by modifying the relative composition of individual membrane phospholipids. Our understanding of regulation of eukaryotic phospholipid synthesis is most advanced in the *S. cerevisiae* system, in which the major phospholipid pathways are subject to a complex network of coordinate control.

Cross-pathway control of the PI, PC, and CL branches of phospholipid synthesis is characterized by the following parameters. (i) The availability of inositol controls expression of phospholipid biosynthetic enzymes in all three branches. Thus, during growth in inositol-containing medium, expression of IPS (27, 34), PSS (13), PSD (125), PEMT (227), PMT (227), and PGP synthase (49) is reduced. Three other phospholipid precursors, choline, ethanolamine, and serine, also affect the expression of some enzymes, to a lesser extent than does inositol. However, their effects are apparent primarily in the presence of inositol (13, 72, 73, 186, 227). (ii) Inositol repression of phospholipid biosynthesis occurs only if cells can synthesize PC. In mutants blocked in de novo synthesis of PC (i.e., *cho2* and *opi3* mutants), expression of the above-mentioned enzymes does not decrease in response to exogenous inositol. However, if the PC pathway mutants are grown in inositol-containing medium to which choline has also been added,

inositol-mediated repression is restored (presumably because the cells can make PC by the Kennedy pathway) (150, 208). (iii) Inositol repression of the PI and PC branch enzymes but not the CL branch is mediated by the *INO2-INO4-OPI1* regulatory circuit. The products of positive regulatory genes *INO2* and *INO4* are required to derepress PI and PC branch enzymes in the absence of inositol, while the *OPI1* negative regulator represses these enzymes in the presence of inositol. Therefore, these enzymes are not expressed in *ino2* or *ino4* mutants (13, 33, 139) and are constitutive in the *opi1* mutant (13, 72, 73, 103, 104). However, these regulatory genes do not affect expression of PGP synthase, the CL branch enzyme (49).

Control by Factors Affecting Mitochondrial Development

There are several reasons why the degree of mitochondrial development has an impact on phospholipid synthesis. First, the elegant work of Barbara Stevens (203) depicting mitochondrial development as a function of cell physiology confirmed that the degree of mitochondrial membrane development depends on physiological conditions and that mitochondrial membrane volume can vary by more than fivefold. Moreover, there is no condition, including anaerobiosis, under which mitochondrial membranes are absent. In fact, it is a general belief that mitochondria, in some form, are essential. Therefore, phospholipid synthesis must be regulated to meet the constantly changing requirement for mitochondrial membrane synthesis.

Second, several phospholipid biosynthetic enzymes are located in the mitochondrial membrane. These include not only the enzymes for synthesis of the mitochondrion-specific phospholipid CL (i.e., PGP synthase, PGP phosphatase, and CL synthase) but also enzymes for general phospholipid synthesis (PSS, PSD1, PIS, CDP-DGS, and choline phosphotransferase) (117). Therefore, the degree of mitochondrial development may also dictate the availability of certain biosynthetic enzymes.

Third, synthesis of the phospholipid precursor PA depends on the availability of G3P and possibly of DHAP, depending on the relative contribution of the two pathways to PA synthesis (as discussed below). The availability of these glycolytic intermediates may be a function of the relative contribution of glycolysis and the tricarboxylic acid cycle to cellular energy production, which in turn is related to the state of mitochondrial development.

Mitochondrial membrane development is favored under respiratory growth conditions, i.e., in the presence of nonfermentable carbon sources such as glycerol or ethanol, in the presence of oxygen, and in the stationary as opposed to logarithmic growth stage in medium containing glucose. As expected, the mitochondrial phospholipid CL is present in greater quantity under respiratory growth conditions (86). This is brought about by increased expression of the first CL pathway enzyme, PGP synthase, which catalyzes the committed step in CL synthesis (44, 94, 210). Increased expression of PGP synthase was apparent in both the presence and absence of inositol.

Activities of the PA biosynthetic enzymes GPAT, DHAPAT, and acyl DHAP reductase were all greater (although to a different extent) during growth in nonfermentable carbon sources than in growth in glucose and fructose (155). All three enzymatic activities increased as wild-type cells grown on glucose entered the stationary phase of growth (155). All three activities were also greater during aerobic than anaerobic growth (188). These data suggest that expression of all three enzymes of PA synthesis is greater under growth conditions which lead to increased mitochondrial development.

In contrast to the PA and CL biosynthetic enzymes, the activity of the PI branch enzyme, PIS, was constitutively elevated, while the activity of CDP-DGS and the PC branch enzymes, PSS, PEMT, and PMT, decreased as cells entered stationary phase in inositol-containing medium (74). Analysis of relative membrane phospholipid composition indicated that PI levels increased at the expense of PS levels in the stationary phase. However, in inositol-containing medium, while PIS activity remained constitutively elevated, CDP-DGS, PSS, PEMT, and PMT activities were constitutively reduced. Therefore, the growth phase response is not independent of inositol regulation. The effects of carbon source and oxygen on the activities of these enzymes has not been reported. Therefore, it cannot be concluded that the change in enzyme activities, as a function of growth phase, can be attributed solely to the respiratory state of the cell.

LEVELS OF REGULATION

For membrane phospholipid synthesis to respond efficiently to changes in respiratory state, inositol concentration, and possibly other physiological signals, several levels of regulation have evolved, and it is not unusual for some enzymes to be regulated at more than one level. Reduction of mRNA steady-state levels in response to inositol has been demonstrated for transcripts of the *INO1* (66), *CHO1* (13), *CHO2* (42, 83, 108), *OPI3* (42, 83, 108), *ITR1* (122), *CKI* (76), and *CTR1* (136, 161) genes and, to a much lesser extent, for transcripts of the *FAS1*, *FAS2*, and *FAS3*/*ACC1* genes (22, 24, 195). For these genes, the transcriptional response to inositol is regulated by the *cis*-acting UAS_{INO} sequence and the *trans*-acting *INO2-INO4-OPI1* regulatory genes, which are discussed more thoroughly in the sections to follow. The list of inositol-responsive genes may expand as the genes encoding other phospholipid biosynthetic enzymes are cloned. For example, CDP-DGS (73) and acyl DHAP reductase (155) showed slight but significantly reduced activity in response to inositol supplementation. GPAT and DHAPAT activities were also slightly reduced under these conditions, although the statistical significance of the decrease is questionable because of the limited sensitivity of the assay in crude extracts (155). The mitochondrial enzyme PGP synthase showed a reduction of activity, which was not dependent on the *INO2-INO4-OPI1* regulatory circuit, in response to inositol (49). More sensitive determinations will be possible once the genes encoding these enzymes are cloned.

Posttranslational modification appears to play a role in the regulation of at least two phospholipid biosynthetic enzymes, PGP synthase and PSS. A drop in the specific activity of PGP synthase is observed within minutes of addition of exogenous inositol and is therefore too rapid an effect to be explained by repression of enzyme synthesis alone (49). This rapid effect is more likely to be the result of inactivation and/or degradation of the enzyme. Recently, an enzyme inactivation-degradation mechanism has been shown to regulate the Itr1 enzyme in response to inositol supplementation (121). The kinetics of PGP synthase repression by inositol differ significantly from those observed by Culbertson et al. (27) for repression of IPS. No decrease in IPS activity was observed until 1 h after the addition of inositol, and the much more dramatic 50-fold repression of IPS activity was not fully apparent until 10 h after the addition. Immunoprecipitation of IPS from cells following addition of inositol revealed no significant decrease in enzyme levels for hours (47). Another enzyme which may be regulated posttranslationally is PSS. Kinney and Carman (98) showed

that the 23-kDa subunit of PSS can be phosphorylated in vitro and in vivo by the cyclic AMP (cAMP)-dependent protein kinase. PSS activity was reduced in the *bcy1* mutant, which has high cAMP-dependent protein kinase activity, and was elevated in the adenylate cyclase mutant *cyr1*, which has low kinase activity, suggesting that enzyme activity is inversely related to phosphorylation. Addition of cAMP to *cyr1* mutant cells results in a decrease in PSS activity and in the relative ratio of PS to PI (97). That phosphorylation of PSS is apparent only in logarithmically growing cells is consistent with observations that the cAMP-dependent protein kinase is inactivated during the stationary phase of growth (147, 148). The physiological relevance of this mechanism is not clear in light of the evidence that PSS activity is greater in logarithmically growing cells (74). The relative contributions of these two mechanisms to the overall regulation of PSS expression remain to be elucidated.

Purification of several phospholipid biosynthetic enzymes has enabled investigation of the effect of phospholipids and phospholipid precursors on enzyme kinetics. Of the enzymes purified, allosteric regulation by inositol has been reported only for PSS (92). However, many enzymes are modified by their lipid environment. In fact, PIS is stimulated by PS, while PSS activity is decreased by an increase in the PI-to-PS ratio (39, 81). Therefore, although PIS is not regulated at the level of synthesis or growth phase, the enzyme itself is regulated allosterically. Since PIS and PSS vie for the common substrate CDP-DG, allosteric regulation of these two enzymes might be critical for overall phospholipid synthesis.

How do these regulatory mechanisms respond to exogenous inositol? In the case of PSS, transcription is repressed and enzyme activity is allosterically inhibited by inositol. As PSS activity decreases relative to PIS, the PI-to-PS ratio increases, which further inhibits PSS activity. When inositol is depleted, transcription is derepressed, enzyme inhibition is removed, and more PS is made. PIS is stimulated by an increase in PS, but perhaps this is just to keep up with the bare minimum activity that the cell needs to meet its PI requirement.

INOSITOL/CHOLINE RESPONSE

As discussed above, the primary metabolic signal that coordinates regulation of phospholipid biosynthetic gene expression is the presence of the soluble precursors inositol and choline in the growth medium. In principle, this response seems reasonable, since the presence of inositol eliminates the need to synthesize it in vivo (Fig. 1). Likewise, the availability of exogenous choline also reduces the need to synthesize PC de novo when it may be energetically more favorable to synthesize it via the salvage pathway (Fig. 1). The molecular details of the response to inositol and choline are becoming clear. In *S. cerevisiae*, coordinated regulatory responses are established by specific *cis*-acting regulatory regions (53, 54, 207). These *cis*-acting elements are conventionally referred to as upstream activation sequences (UAS) and function as binding sites for transcriptional activator proteins (54, 207). The regulation of phospholipid biosynthetic gene expression has been shown to be dependent on a promoter element (UAS_{INO}) which is the binding site for a transcriptional activator complex composed of Ino2p and Ino4p. In the sections that follow, we will describe the evidence that led to these conclusions.

The promoters of some yeast genes have also been shown to include sequences required for repression of transcription (16, 134). The sequences are frequently called upstream repressor sequences (URS) and serve as binding sites for transcriptional repressor proteins (202). We will review the molecular genetic evidence that transcription of the *INO1* gene is under control of a URS in addition to the UAS_{INO}. We will also discuss the properties of two global transcriptional repressors, Sin3 and Ume6, that function to repress phospholipid biosynthetic gene expression.

UAS_{INO} Regulatory Sequence

As is the case with most yeast genes, coordinated regulation of phospholipid biosynthetic gene expression is brought about by a common *cis*-acting regulatory element called a UAS (53, 54, 207). In general, UAS elements serve as binding sites for transcriptional activator proteins which specify transcription in response to a specific signal. In the case of the phospholipid biosynthetic genes, the response to inositol and choline in the growth medium is dictated by a 10-bp element called the UAS_{INO}. As discussed above, at least one copy of the UAS_{INO} element has been identified in the promoters of all genes that are responsive to inositol and choline.

The identification of the UAS_{INO} element resulted from a strategy that included promoter deletion studies (described above) and DNA sequence inspection (12, 20, 65, 141). Sequence inspection of the promoters of 23 genes that are responsive to inositol and choline initially identified a commonly repeated 9-bp sequence with the consensus 5′ ATGTGAAAT 3′ (20). The promoter deletion experiments showed that this 9-bp sequence was necessary for the inositol/choline response. However, insertion of four copies of an oligonucleotide that contained the 9-bp consensus sequence upstream of a UAS-less *CYC1-lacZ* reporter gene did not activate expression of this reporter gene (141). Thus, the nonamer element was necessary but not sufficient for UAS_{INO} function.

The identity of the UAS_{INO} element was established by analysis of several restriction fragments from the *INO1* (141) and *CHO1* (12) promoters that stimulated transcription of the *CYC1-lacZ* reporter gene. These experiments revealed that every restriction fragment that conferred inositol-specific regulation to the reporter gene contained the nonamer sequence flanked by a C residue at the 5′ position. The possibility that UAS_{INO} function depends on a 10-bp sequence with a C at the first position was tested formally by inserting an artificial oligonucleotide upstream of the *CYC1-lacZ* reporter gene (11). This experiment revealed that the UAS_{INO} element is the 10-bp sequence 5′ CATGTGAAAT 3′ (11), which is sufficient for the inositol/choline response. The sequence requirements for each nucleotide of the UAS_{INO} element, as well as 5′-flanking nucleotides, were tested in vivo with the *CYC1-lacZ* reporter gene (11). These studies show that substitutions of the first 6 bases (5′ **CATGTG**AAAT 3′) of the UAS_{INO} element either completely eliminate or severely reduce its function (11). The UAS_{INO} element serves as a binding site for a heterodimer of the Ino2p and Ino4p proteins (4, 174), which belong to the helix-loop-helix (HLH) family of proteins (80, 173, 175). The strong sequence requirement for the first 6 bases of the UAS_{INO} element is not surprising, since the general binding site for HLH proteins (5′ CANNTG 3′) (14) is contained within the first 6 nucleotides of the UAS_{INO} element. The 2 bases immediately flanking (i.e., preceding) the UAS_{INO} element the seventh and eighth positions (5′ CATGTG**AA**AT 3′) of the UAS_{INO} element are also important for UAS function (11). Substitutions of the final 2 bases (5′ CATGTGAA**AT** 3′) have less dramatic effects (11).

The identification of the UAS_{INO} element is corroborated by reports (108, 110) that describe inositol/choline-responsive UAS elements that are strikingly similar to the UAS_{INO} element. These studies examined the *PEM1* (*CHO2*), *PEM2*

TABLE 2. Genes that contain putative UAS_{INO} elements in their promoters

ADK1	*CTR1*	*FAS2*	*ITR2*	*PSD1*
ATP2	*CYP51*	*FAS3*	*MFA1G*	*PYK1*
CCT1	*EPT1*	*FPP1*	*OLE1*	*SLC1*
CHO1	*ERG1*	*INO1*	*OPI3*	*SPE2*
CHO2	*ERG3*	*INO2*	*PHO5*	*Ty1-H3*
CKI1	*ERG12*	*INO4*	*PIS1*	*Ty3-1*
CPT1	*FAS1*	*ITR1*	*PMA1*	

(*OPI3*), and *PSS1* (*CHO1*) promoters and identified an octamer regulatory sequence, 5′ CATRTGAA 3′. It is noteworthy that this octamer sequence is identical to the first 8 bp of the 10-bp UAS_{INO} element. However, artificial oligonucleotides that included this 8-bp sequence, and therefore deviated from the UAS_{INO} consensus at the last two positions, were able to activate transcription of the *CYC1-lacZ* reporter gene only modestly. An ICRE (also called UAS_{FAS}) was identified in the promoters of the *FAS1*, *FAS2*, and *FAS3* (*ACC1*) genes (22, 24, 195). Studies of the promoters of these genes identified the ICRE as an 11-bp sequence (5′ TYTTCACATGY 3′). Clearly, the first 10 bp of the ICRE are complementary and inverted relative to that of the UAS_{INO} element. Since the UAS_{INO} element has been shown to be functional in both orientations (141), the ICRE and the UAS_{INO} are likely to be the same element. Collectively, the experiments on the *PEM1* (*CHO2*), *PEM2* (*OPI3*), *PSS1* (*CHO1*), *FAS1*, *FAS2*, and *FAS3* (*ACC1*) genes support the conclusion that the inositol/choline response is mediated through a 10-bp UAS_{INO} element.

The aforementioned studies also revealed that the UAS_{INO} element is not restricted to genes directly involved in phospholipid biosynthesis. In fact, potential UAS_{INO} elements have been observed in the promoters of 34 genes (Table 2). However, it has yet to be determined if all of these genes (Table 2) are regulated in response to inositol. We already know that some genes, such as *PIS1* and *INO4*, are not regulated in response to inositol (discussed above). Consequently, the presence of a UAS_{INO} element in a promoter does not necessarily imply that the UAS_{INO} element will be functional. Meanwhile, a comparison of the 123 potential UAS_{INO} elements also provides clues to sequence requirements (Table 3). This sequence comparison reveals a strong conservation of the 10-bp sequence. It is also interesting that several genes (e.g., *ADK1*, *MFA1G*, *PYK1*, *PMA1*, and *PHO5*) with no direct link to phospholipid synthesis are regulated in response to inositol and are sensitive to mutations in the *INO2* and *OPI1* regulatory genes (179).

It is also noteworthy that while all yeast genes that are responsive to inositol and choline have an identifiable UAS_{INO} element in their promoters, not all yeast promoters containing UAS_{INO} elements are inositol/choline responsive. For example, the promoters of the *CTR1* and *INO4* genes all have identifiable UAS_{INO} elements that are not inositol/choline responsive (6, 136). Curiously, expression of the *INO4-cat* gene requires *INO4* but not *INO2* (6). Another function that requires the *INO4* gene product but not the *INO2* gene product is the sphingolipid biosynthetic enzyme, IPC synthase (107). IPC synthase activity is stimulated by growth in the presence of inositol, and this induction requires the *INO4* gene. Since Ino4p does not have the capacity to homodimerize or activate transcription (4, 197), these observations suggest that there are alternate partners for Ino4p. Even though yeast HLH proteins have yet to be shown to form heterodimers with multiple partners, this is a recurring theme among mammalian HLH proteins (3, 223).

TABLE 3. Compilation of potential UAS_{INO} elements

Nucleotide	No. of elements with nucleotide at position:									
	1	2	3	4	5	6	7	8	9	10
C	**86**	5	4	5	7	3	7	6	3	4
A	18	**100**	13	16	13	10	**103**	**99**	**84**	31
T	8	12	**104**	9	**99**	34	6	8	29	**82**
G	11	6	2	**93**	4	**76**	7	10	7	6
Consensus	C	A	T	G	T	G	A	A	A	T

INO2 and *INO4* Positive Regulatory Genes

The *INO2* and *INO4* genes encode positive regulators of transcription that are required for derepression in response to inositol and choline deprivation (13, 66, 105, 175). These two genes were initially identified among the original 10 inositol auxotrophic complementation groups (29, 33). This inositol growth requirement is the same as that of *ino1* mutant strains because *ino2* and *ino4* mutant strains fail to express the *INO1* gene product (34). The prediction that these two genes encoded regulators of phospholipid biosynthesis, rather than structural genes, was further supported by their global effects on phospholipid biosynthesis. That is, mutant alleles of these genes had pleiotropic effects on the PI as well as the PC branches of the phospholipid biosynthetic pathways (139). More recently, these genes have been implicated in the expression of a number of biochemical pathways, including fatty acid synthesis (24, 196), inositol transport (122), nuclear segregation (58), and bud pattern formation (58). It is now clear that the *INO2* and *INO4* genes have far-ranging roles beyond the control of membrane synthesis.

The inositol requirement of *ino2* and *ino4* mutant strains proved particularly useful in the cloning of complementing genes. That is, both the *INO2* and *INO4* genes were cloned by their ability to complement the inositol growth requirement, which restored expression of the *INO1* gene product (105, 175). A combination of genetic and molecular strategies identified a 453-bp open reading frame in the *INO4* clone, whereas the *INO2* coding sequence is contained in a larger, 912-bp open reading frame. The identification of these open reading frames made it possible to speculate about the structure-function relation of these two proteins. For example, the Ino2p and Ino4p predicted protein sequences share similarity to the *myc* family of mammalian oncogenes (80, 175). Specifically, the highest degree of similarity is in the HLH domain of the *myc* oncogenes (Fig. 2). This observation is striking since the *INO2* and *INO4* gene products form a heterodimer, and the HLH domain is recognized as a protein dimerization domain (158).

The prediction that Ino2p and Ino4p form a heterodimer that binds DNA is suggested by mobility shift experiments which identify protein-DNA complexes that assemble with the *INO1* promoter (140). The formation of these complexes is dependent on wild-type alleles of both the *INO2* and *INO4* genes. More recently, the nature of the interaction between Ino2p and Ino4p and DNA has been established at a biochemical level. Experiments with antibodies specific to Ino2p have shown that the *INO2* gene product is present in the previously defined protein-DNA complexes (174). Furthermore, the same protein-DNA complexes are formed by using Ino2p and Ino4p synthesized in *E. coli* (4, 197). In the latter situation, the Ino2p-Ino4p heterodimer was found to form independently of the

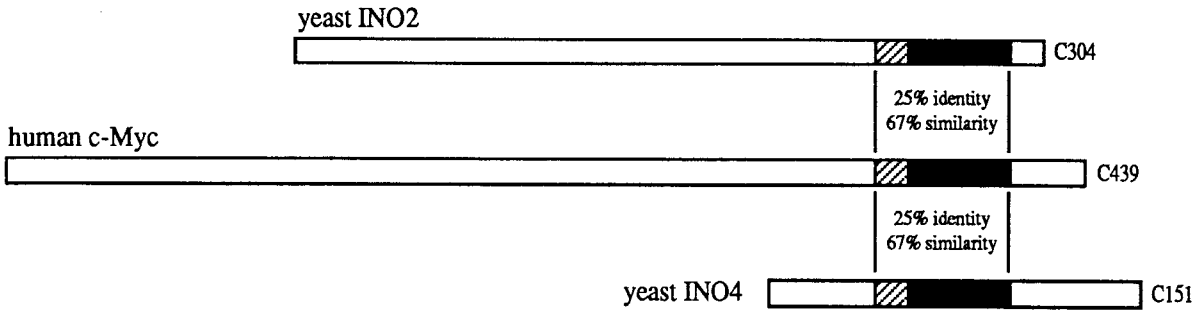

FIG. 2. Schematic comparison of the amino acid sequence of the yeast Ino2 and Ino4 transcriptional activator proteins with that of the human c-Myc oncoprotein. The locations of the basic (▨) and helix-loop-helix (■) regions are noted.

UAS_{INO} element (4). A requirement for the HLH domain in Ino2p-Ino4p dimerization has been shown by using the two-hybrid system and far-Western analyses (197). Therefore, the formation of an Ino2p-Ino4p heterodimer and its assembly with the promoter sequences in the *INO1* gene are supported by genetic, molecular, and biochemical experiments. The specificity of the interaction between Ino2p-Ino4p and DNA has been further resolved by demonstrating that the heterodimer, synthesized in vitro, specifically binds to an artificial consensus UAS_{INO} element but not a UAS_{INO} element that deviates from the consensus (4). Therefore, binding of the Ino2p-Ino4p heterodimer to the UAS_{INO} element is sequence specific. The transcriptional activation function is dependent on two regions of the amino terminus of Ino2p, whereas Ino4p does not have the ability to activate transcription (197). Thus, Ino2p is the transcriptional activator, but its ability to bind the UAS_{INO} element is dependent on dimerization with Ino4p.

OPI1 Negative Regulatory Gene

The *OPI1* gene product is required for repression of phospholipid biosynthetic gene expression in response to inositol and choline supplementation. The *OPI1* gene was initially defined by using a bioassay for mutants that excrete inositol into the growth medium (51). While it is not clear why *opi1* mutant strains excrete inositol, the phenotype is very probably due to the overproduction of the *INO1* gene product. Immunoprecipitation experiments with anti-IPS revealed that the *INO1* gene product is constitutively overproduced in an *opi1* mutant strain regardless of the growth conditions (48, 103). Moreover, the overproduction of the IPS subunit must elevate the intracellular inositol levels, since *opi1* mutants have a higher percentage of PI in their membranes. In fact, the level of PI in the membranes of *opi1* mutant strains is equivalent to that of wild-type strains grown in the presence of 50 μM inositol (103). However, the phenotype of the *opi1* mutants is not restricted to the *INO1* gene product. Strains harboring *opi1* mutant alleles also display constitutive overexpression of the PSS activity and altered levels of the PC biosynthetic enzyme activities (103). Thus, the *OPI1* gene has a pleiotropic effect on several phospholipid biosynthetic enzyme activities.

Our understanding of a molecular function for the *OPI1* gene product is based on the effect of *opi1* mutants on transcription of the phospholipid biosynthetic genes. That is, the constitutive overproduction of the IPS and PSS subunits in the *opi1* mutant strains is due to a defect in transcriptional regulation of the *INO1* and *CHO1* genes, respectively (13, 66). Northern (RNA) blot analyses revealed that the *INO1* and *CHO1* transcripts are constitutively overexpressed in an *opi1* mutant strain, and, more recently, the *CHO2* and *OPI3* transcripts have also been observed to be constitutively overexpressed (84). This effect on *CHO2* and *OPI3* transcription suggests that the altered pattern of PC synthesis in the *opi1* mutant strains is attributable to a defect in transcriptional regulation.

An examination of the Opi1p predicted protein sequence identified two features that all hallmarks of transcriptional regulatory proteins, namely, a leucine zipper and glutamine-rich regions (224). The leucine zipper domain was originally identified as a dimerization domain in the mammalian C/EBP protein transcriptional regulatory protein (126). Dimerization of the C/EBP protein is a prerequisite for formation of protein-DNA complexes, since it juxtaposes two basic domains that dictate interaction with DNA (127). However, it is presently not known if Opi1p forms a dimer or if it interacts directly with DNA. The glutamine-rich regions of the *OPI1* gene are particularly noteworthy, since such domains have been observed in a number of transcriptional regulatory proteins and are likely to be involved in protein-protein interactions (45). Alterations of these domains, specifically amplifications and deletions, have profound effects on protein function. For example, it has been shown that amplifications of trinucleotide repeats (some encoding glutamines) are the cause of Kennedy's disease in humans (118, 189). Conversely, deletion of polyglutamine-rich domains from the mammalian SP1 transcriptional activator has negative effects on its ability to interact with coactivators (182).

Despite the aforementioned observations, it is not clear how Opi1p specifically functions to control phospholipid biosynthetic gene expression. However, it seems likely that Opi1p is a key player in the response to inositol. The identification of superrepressor mutant alleles, such as the ones which have been described for the *GAL80* (177) gene, would certainly help to define the function of Opi1p. One aspect of the function of Opi1p that is clear is that it regulates phospholipid biosynthesis through the UAS_{INO} element. This conclusion is based on the observation that *opi1* mutants constitutively overexpress a *CYC1-lacZ* heterologous reporter gene under the control of the UAS_{INO} element (11). However, these data simply demonstrate that Opi1p does not use another *cis*-acting promoter element (such as a URS element) but do not provide evidence for a direct interaction between Opi1p and the UAS_{INO} element. There was the possibility that the ability of Opi1p to regulate *INO1* transcription through the UAS_{INO} element involved regulating transcription of the *INO2* activator gene (6). However, *INO1* expression is not responsive to inositol in an *opi1* mutant strain when *INO2* is expressed by using the *GAL1* promoter (7). Therefore, Opi1p must regulate *INO1* expression directly.

On the basis of our current understanding of the *opi1* mutant phenotype and recognizable protein domains of Opi1p

and by analogy to other repressor-regulated systems, we can formulate a hypothetical model. Our model predicts that Opi1p might interact transiently with either Ino2p or Ino4p. This model is reminiscent of the systems that regulate the *GAL* (133) and *PHO* (87) genes. We favor this model because the formation of the Ino2p-Ino4p-UAS_{INO} complex is not dramatically disrupted under repressing growth conditions (140). Therefore, it does not appear that Opi1p precludes the formation of this complex by reducing expression of Ino2p and Ino4p or by competing for the UAS_{INO} element. Moreover, the existence of the glutamine-rich domains suggests potential protein-protein interactions. In support of this, all of the *opi1* mutants that have been sequenced predict truncated Opi1p lacking the polyglutamine-rich domain (224). Clearly, a formal examination of this model (and other potential models) will have to be carried out.

Other Regulatory Genes

Two mutants that affect expression of the *INO1* gene have been described. The first mutant is a dominant inositol auxotroph, which is designated *CSE1* (77). Strains which harbor this mutation are unable to derepress expression of the *INO1* gene or its product. Moreover, this mutation also creates a novel situation, in that *INO1* expression is sensitive to choline. Normally, *INO1* expression is sensitive to choline only in combination with inositol (66). The precise molecular details of why this mutation misregulates *INO1* expression are not known. However, it is known that the mutation is not linked to the *INO1* gene itself and that *INO2* (*SCS1*) is a multicopy suppressor of the inositol auxotrophy (78). The *CSE1* mutant phenotype is also suppressed by the *SCS2* gene, which encodes a predicted protein of 244 amino acids (163). The *SCS2* gene product has been implicated in regulation of *INO1* expression; however, its precise function is not known (163).

Another mutant, the *dep1* mutant, was identified by its inability to fully repress expression of an *INO1-lacZ* fusion reporter when grown on media supplemented with either inositol or inositol plus choline (124). Strains that harbored the *dep1* mutant allele are defective in derepression and repression of the *INO1*, *CHO1*, and *OPI3* genes. The *dep1* mutant was mapped to the left arm of chromosome I (12 centimorgans from the centromere), which contains several open reading frames of unknown function.

The *IRE1* gene is also required for regulation of *INO1* gene expression (172). Strains that carry disruption alleles of the *IRE1* gene require inositol for growth (172). The sequence of the *IRE1* gene predicts a protein product of 127 kDa with two highly hydrophobic regions (amino terminus) and sequence similarity to the catalytic domains of protein kinases (carboxy terminus) (172).

Autoregulation of *INO2* Expression

While the *cis*- and *trans*-acting regulatory components required for regulation of phospholipid biosynthetic genes in response to inositol and choline are now recognizable, the mechanism of regulation has yet to be determined. Examination of the molecular and genetic properties of the *trans*-acting factors suggests that protein-protein interactions may play an important role in repressing transcription of the phospholipid biosynthetic genes. For example, the inositol/choline response may involve a mechanism such as the ones that regulate expression of the *GAL* (88, 133) and *PHO* (87) genes. Activation of *GAL* gene expression requires Gal4p, which is bound to the UAS_{GAL} element (55, 89). However, Gal4p is always in a complex with the repressor protein Gal80p (133), which copurifies with Gal4p through three chromatographic columns (181). Under noninducing conditions (growth in glycerol), the interaction between Gal4p and Gal80p is altered to allow for Gal4p-mediated transcriptional activation of the *GAL* genes. Similarly, *PHO5* expression is regulated by an interaction between Pho4p (positive regulator) and Pho80p (negative regulator) (87). Regulation of phospholipid biosynthesis may involve a similar interaction between the Ino2-Ino4p heterodimer and Opi1p.

Another common mechanism for regulating gene expression in *S. cerevisiae* is to modulate activator protein levels. For example, general control of amino acid biosynthesis is determined by varying the translation and protein stability of the cognate activator protein, Gcn4p (64, 115). While the amount of *GCN4*-encoded mRNA is constant, the protein level is regulated at the translational level by short open reading frames in the 5′ leader of the *GCN4* transcript (64). Repression of the structural genes for galactose utilization in response to glucose (catabolite repression) is also partly established by repressing expression of the *GAL4* activator gene (52, 123). Consequently, it is possible that a mechanism for regulation of phospholipid biosynthetic gene expression involves controlling the levels of the activators or repressors. This possibility is supported by the observation that the promoters of the *INO2* and *INO4* genes include copies of the UAS_{INO} element (6, 196). Moreover, mobility shift assays with extracts from cultures grown under derepressing conditions yielded more Ino2p-Ino4p-UAS_{INO} complex than did extracts from cultures grown under repressing conditions (140).

Transcriptional regulation of the *INO2* and *INO4* genes was examined by fusing the promoters of these genes to the *cat* reporter gene (6). Each of the fusion genes was assayed from single integrants at the *GAL4* locus to allow for comparison of the relative rates of expression of these genes. The results reveal that *INO2* expression is induced 10-fold in the absence of inositol and choline (6). Moreover, *INO2-cat* expression is dependent on the *INO2* and *INO4* genes and is constitutively overexpressed in an *opi1* mutant strain. Thus, the pattern of *INO2* expression is identical to that of one of its target genes, *INO1* (66). Conversely, the *INO4-cat* gene is constitutively expressed under all growth conditions. This result is in disagreement with that of another study, which found that an *INO4-lacZ* fusion gene is regulated in response to inositol supplementation (196). However, the level of *INO4-lacZ* expression that was reported was actually lower than the level of *lacZ* expression in the absence of the *INO4* promoter. The *INO4-cat* experiments also showed that expression from the *INO4* promoter is dependent on a wild-type *INO4* gene but not a wild-type *INO2* gene (6). This observation suggests that there may be another partner for Ino4p which is required for constitutive expression of the *INO4* gene. The results further show that *INO4-cat* is overexpressed relative to *INO2-cat*, which suggests that Ino2p may be limiting relative to Ino4p. This prediction is supported by experiments which show that the presence of the *INO2* (*SCS1*) gene on a multicopy plasmid results in higher than normal *INO1* expression under repressing conditions (78) and leads to increased formation of an Ino2p-Ino4p-UAS_{INO} complex (174).

Collectively, the aforementioned studies raise the possibility that regulation of phospholipid biosynthetic gene expression is brought about by regulating transcription of the *INO2* activator gene. This possibility has been examined by studies with a strain that contained the *INO2* gene under the control of the *GAL1* promoter (7). In this strain, *INO2* transcription is regulated by the carbon source but not by inositol (7). These studies show that *INO1* and *CHO1* gene expression is sensitive

to inositol, even though *INO2* transcription is not. However, the level of *INO1* and *CHO1* transcription is dependent of the level of *INO2* transcription. Therefore, the purpose of regulating *INO2* expression is not to regulate the expression of its target genes, in response to inositol, but to determine the level of target gene expression (6, 7).

URS1^{INO1} Regulatory Sequence

The expression of some yeast genes has been shown to be controlled by repressors that specifically interact with promoter sequences called URS elements (16, 134). For example, catabolite repression of the yeast *GAL1* gene is established by URS elements that are found in the promoters of the *GAL1* and *GAL4* genes (40, 52). Several other systems have been shown to be under control of a URS element, namely, the *HO* (202), *CAR1* (144), and *SPO13* (18) genes. For these three genes, repression is effected by a common regulatory sequence generally called the URS1 element (5′ AGCCGCCGA 3′) (18, 144, 202). However, the function of the URS1 element is not likely to be limited to these three genes, since a substantial number of yeast promoters have sequence elements that resemble the URS1 (144).

One promoter that contains a URS1 element directs transcription of the *INO1* phospholipid biosynthetic gene (142). The URS1^{INO1} element is a perfect match to the consensus sequence. Deletion of the URS1^{INO1} element from the *INO1* promoter results in a substantial increase in the expression of reporter genes regardless of the growth conditions (84, 142). Consequently, the URS1^{INO1} element does function to generally repress expression of the *INO1* gene. It is tempting to speculate that the URS1^{INO1} element may account for differences between regulation of *INO1* expression and regulation of the other phospholipid biosynthetic genes in response to inositol. For example, the pattern of regulation of *INO1* expression is unusual in that the repressed level of expression is dramatically lower than the level of expression of the coregulated genes. Consistent with this prediction, deletion of the URS1^{INO1} element does raise the repressed level of *INO1* expression to a level equivalent to that of other phospholipid biosynthetic genes (84, 142, 199).

SIN3 and *UME6* Global Negative Regulators

The ability of the URS1 element to repress gene transcription is dependent on the products of the *SIN3* and *UME6* genes (83, 84). That is, the expression of some genes that have URS1 elements in their promoters is sensitive to mutations in either the *SIN3* gene (206, 219), the *UME6* gene (180), or both (206). For example, repression of *HO* expression has been shown to be dependent on the *SIN3* gene, although it does not appear that the product of the *SIN3* gene directly interacts with the *HO* promoter (220, 222). Likewise, expression of the early meiotic gene, *SPO13*, is affected by mutations in both the *SIN3* (*UME4*) and *UME6* genes (205, 206, 219). However, expression of the *CAR1* gene is regulated by the *UME6* gene but not the *SIN3* gene (180). Consequently, these repressor proteins are able to interact in different combinations to bring about repression of gene expression. The roles of Sin3p and Ume6p appear even more complex when one examines how they interact with the URS1 element. For instance, evidence suggests that Ume6p interacts directly with URS1^{SPO13} element (206). This observation is consistent with the predicted C6 zinc cluster domain in the *UME6* open reading frame, similar to that of the Gal4p DNA-binding domain. However, UME6-mediated repression of the *CAR1* gene is brought about by interacting with the Buf1-Buf2-Buf3 trimeric complex that directly binds the URS1^{CAR1} element (143). Likewise Sin3p-mediated repression of *HO* gene expression requires a protein, Sdp1, that recognizes the URS1^{HO} element (221). These observations suggest that the Sin3p and Ume6p repressors function to repress transcription of a diverse set of genes in *S. cerevisiae* but may do so by different mechanisms.

Since both Sin3p and Ume6p function through the URS1 element, it is not surprising that they were identified by a number of different genetic screens. For example, the *SIN3* gene was defined by defects in *HO* gene expression (202), early meiotic gene expression (*UME4*) (205, 219), potassium uptake (*RPD1*/*SDI1*) (219), extracellular glucoamylase production (*GAM2*) (229), and, most recently, *INO1* expression (*CPE1*) (83). The defect in *INO1* expression was identified by a genetic screen for mutants that expressed an *INO1-lacZ* reporter gene under repressing conditions. Three different mutants were isolated by using this screen and shown to be allelic (designated *cpe1*, for constitutive phospholipid biosynthetic gene expression). It is now known that all of these mutants (*sin3*, *ume4*, *rpd1*, *sdi1*, and *cpe1*) are allelic to each other and represent the same genetic locus. Consequently, the *cpe1* designation has been changed to *sin3*, since this is the original name for mutations at this locus. As was the case for *SIN3*, the *UME6* gene was also identified by defects in two distinct biological processes, early meiotic gene expression and arginine catabolism (*CAR80*) (206, 226).

The role(s) of the *SIN3* and *UME6* repressor genes in expression of the phospholipid biosynthetic genes has been examined by two different but related strategies. First, the effect of mutations at these two loci on phospholipid biosynthetic gene expression was quantitated by Northern blot hybridization. Surprisingly, mutations in either gene affect expression of the phospholipid biosynthetic genes pleiotropically (83, 84). It is surprising to find that expression of all of the phospholipid biosynthetic genes tested (*INO1*, *CHO1*, *OPI3*, and *CHO2*) is affected, since only the *INO1* promoter has a recognizeable URS1 element (83). The second strategy was to determine the effect of a *sin3* null mutant allele on expression of various fusions of the *INO1* promoter region to the *CYC-lacZ* reporter gene (199). Basically, three types fusions were tested: some included the URS1^{INO1} element and the UAS$_{INO}$ elements; some included the UAS$_{INO}$ elements but lacked the URS1^{INO1} element; and some used the artificial consensus UAS$_{INO}$ element. These studies clearly demonstrate that the *sin3* mutant allele affects expression from both the sole URS1^{INO1} element and the UAS$_{INO}$ elements. These results also explain the pleiotropic effect on expression of phospholipid biosynthetic genes that lack the URS1 element but include the UAS$_{INO}$ element. At present, it is not clear how the Ume6p and Sin3p repressors function through the UAS$_{INO}$ element; however, it seems reasonable that they would exert the greatest effect on *INO1* expression, since the *INO1* promoter includes both the UAS$_{INO}$ elements and the URS1 element. This observation may explain the most obvious difference between *INO1* expression and that of the other phospholipid biosynthetic genes, namely, the significantly lower level of *INO1* expression under repressing conditions.

INO1 TRANSCRIPTION AND THE GENERAL TRANSCRIPTION MACHINERY

In the process of analyzing the *INO1* promoter for *cis*-acting elements required for its expression and regulation, it was observed that the *INO1* gene could be expressed in the absence of UAS$_{INO}$ element independently of the Ino2p activator (65, 141, 209). These observations suggest that expression of the

INO1 gene under these circumstances is probably brought about by the basal transcription machinery. Consistent with this observation, it is particularly noteworthy that perturbations of genes which affect chromatin and nucleosome structure (*SWI* and *SIN1* genes) and perturbations of components of the general transcription machinery (TATA-binding protein and subunits of RNA polymerase II) alter the pattern of *INO1* expression (5, 184, 191). While it is not clear why *INO1* expression is so exquisitely sensitive to these defects, it is likely that the effects on *INO1* transcription have to do with control of basal transcription. Consequently, we will review the current understanding of the roles of these various genes in basal transcription.

It is noteworthy that mutations in several proteins involved in basal transcription yield an inositol auxotrophy. For example, it has been shown that strains which harbor mutations in the two largest subunits of RNA polymerase II (RNAP II) are inositol auxotrophs in addition to being heat and cold sensitive for growth (192, 193). Curiously, a specific subset of these mutations are truncations of the essential carboxy-terminal domain (CTD) of the largest subunit (178). The consequence of truncating the CTD from 27 copies to 10 to 12 copies is that *INO1* expression cannot be derepressed in response to inositol deprivation (191). The role of the CTD in transcription is the subject of intense investigation. Several lines of evidence suggest that the CTD is required for the response to transcriptional activators (96, 113, 114). Recent reports have identified a complex of proteins (Srb2, Srb4, Srb5, Srb6, TFIIF, and TFIIH) that is associated with RNAP II and is required for activated transcription by RNAP II (96, 113, 114). One of the components of this complex is the *SRB2* gene product, which is believed to interact with the CTD since *srb2* mutants suppress the inositol auxotrophy associated with the CTD truncations (178). Interestingly, strains harboring null alleles of *srb2* are also inositol auxotrophs (178).

In addition to the defects in the RNAP II holoenzyme, there are defects in other basal transcription factors that have deleterious effects on *INO1* expression. For example, single-base mutations in the gene that encodes the yeast TATA-binding protein (*SPT15*) have been shown to result in inositol auxotrophy (5). Specifically, two mutant forms of TATA-binding protein, which do not bind DNA in vitro, are defective in derepression of the *INO1*, *GAL1*, and *GAL10* genes but have no effect on regulation of the *HIS4* gene (5). Mutations in the *SPT7* gene are also defective in derepression of *INO1* expression (41).

Another set of interesting mutants that are inositol auxotrophs are the *swi1*, *swi2*, and *swi3* mutants. The *SWI* genes were initially identified as positive regulators of *HO* gene expression (185). Mutations in these genes lead to a growth requirement for inositol that is similar to that caused by the CTD deletions. Strains that carry mutant alleles of the *SWI* genes are unable to derepress expression of the *INO1* gene (109, 185). This requirement for inositol can be suppressed by a mutation in the *SIN1* gene which, interestingly, also suppresses the inositol auxotrophy caused by CTD deletions. Recently, it has been shown that the Swi proteins are part of a large complex that also includes the Snf5 and Snf6 proteins (183). It has been proposed that this complex of proteins assists transcriptional activator proteins in relieving chromatin-dependent transcriptional repression (135, 183, 184). Collectively, the data show that *INO1* transcription is exquisitely sensitive to perturbations in several components of the basal transcription machinery that are required for response to transcriptional activators. Consequently, inositol auxotrophy has become a hallmark of defects in the machinery required for basal and activated gene expression in *S. cerevisiae.*

REGULATION OF PHOSPHOLIPID BIOSYNTHESIS IN OTHER FUNGI

Schizosaccharomyces pombe

The fission yeast *Schizosaccharomyces pombe* is an interesting eukaryotic organism from the standpoint of phospholipid synthesis in that no de novo biosynthetic pathway exists for the synthesis of inositol (152). Therefore, as an inositol-requiring organism, it offers an interesting comparison with *S. cerevisiae*, in which inositol plays a crucial role in general phospholipid metabolism. The importance of inositol to eucaryotic cells is underscored by the phenomenon of inositol-less death, which ensues when inositol auxotrophs are starved for inositol (29, 61, 71, 85, 204, 211). Inositol-less death occurs in *S. pombe* also (153, 154). However, the degree of loss of viability is strain and cell concentration dependent and varies with temperature and carbon source (154). Therefore, with the appropriate strains and conditions, inositol-less death provides an extremely powerful enrichment for the identification of spontaneous mutants in this yeast without the necessity of introducing mutations for inositol auxotrophy into starting strains. While it is not clear why some strains of *S. pombe* show greater resistance to inositol-less death, clues were provided by Fernandez et al. (37). In contrast to *S. cerevisiae*, cells of at least one *S. pombe* strain responded to inositol starvation by greater flexibility in altering PI and PS levels and by reutilizing excreted inositol.

Mutants with mutations in the *S. pombe* PC pathway have been identified by screening for choline auxotrophs (63). While PSS mutants were not recovered, complementation analysis of choline auxotrophs identified clones encoding the two methyltransferases (90a). The genes contained in these clones have been named *CHO2* (PEMT) and *CHO1* (PMT) (90a).

Exogenous inositol affects the expression of PGP synthase, PIS, and PSS but not CDP-DGS in *S. pombe* (43, 91). The first three of these enzymes, but not CDP-DGS, are regulated by growth phase as well. Thus, PIS in *S. pombe*, in striking contrast to the *S. cerevisiae* enzyme, appears to be highly regulated. Karkhoff-Schweizer et al. (91) showed that starvation for inositol resulted in increased PGP synthase activity. PC synthesis appeared to be involved in regulation of PGP synthase. Thus, starvation of choline auxotrophs for choline in the presence of inositol led to a transient derepression of PGP synthase. Starvation of choline auxotrophs for both inositol and choline resulted in less PGP synthase derepression than did starvation for inositol alone. Thus, in *S. pombe*, as in *S. cerevisiae*, PGP synthase is regulated by inositol. This regulation is dependent on a functional PC pathway.

Clearly, some mechanisms of regulating phospholipid synthesis in response to environmental changes are conserved, such as the role of inositol in regulation and alterations in the PI-to-PS ratios. A better understanding of the molecular mechanisms used by *S. pombe* to regulate phospholipid biosynthesis may delineate important and conserved functions in eukaryotic phospholipid synthesis.

Candida albicans

As in *S. cerevisiae*, inositol and choline are important regulators of phospholipid synthesis in *Candida albicans* (101). However, one important difference between the two yeasts is that choline alone regulates the methylation pathway in *C. albicans*, while in *S. cerevisiae*, choline regulation is apparent

only in the presence of inositol. The *C. albicans* gene *CaINO1*, encoding IPS, is regulated by inositol at the level of mRNA abundance (100). Sequence analysis reveals 64% identity and 77% similarity to the *S. cerevisiae INO1* gene (106). It is not surprising, therefore, that the *Candida* gene can complement *S. cerevisiae ino1* mutants.

Neurospora crassa

The analysis of phospholipid regulation in *Neurospora crassa* has been neglected of late, although earlier studies were interesting and informative. Choline and inositol auxotrophs, as well as sphingolipid mutants, have been described (82, 130, 204). Inositol-less death has been characterized as an enrichment for spontaneous mutants (204). Hubbard and Brody (82) showed that although this fungus tolerates enormous variations in phospholipid composition, several factors were constant. These included the composition of CL, total phospholipids, total zwitterionic phospholipids, total anionic phospholipids, and the ratio of zwitterionic to anionic phospholipids. These data suggested that phospholipid composition was regulated to maintain constancy of charge. While this organism is less tractable than *S. pombe* and *S. cerevisiae* with regard to molecular analysis, it is an attractive system in which to study morphology and differentiation. The role of phospholipid synthesis in these processes merits additional investigation.

CONCLUSIONS

Our understanding of the genes required for biosynthesis of membrane phospholipids in *S. cerevisiae* has increased dramatically over the last 25 years. Clearly, most of the structural genes have been cloned and sequenced, and current efforts will undoubtedly identify the remaining structural genes. The more recent years have also yielded a considerable amount of information on the regulation of expression of the structural genes in response to inositol. We decided to focus this article on this response because the expression of most of the structural genes is subject to a coordinated response to inositol. In addition, much effort has been devoted to understanding the mechanism that controls this response. Thus, we have reviewed the literature concerning the promoter elements and the cognate regulatory proteins that are required for the response to inositol. The following important questions (among others) remain to be addressed. How many genes are under this coordinate control? What is the intracellular signal that coordinates the response? Which regulatory protein(s) senses the signal? What is the molecular mechanism for the inositol response? These questions are sure to be addressed in the near future, as they are the driving force of investigations in several laboratories.

ACKNOWLEDGMENTS

We acknowledge members of our laboratories, Susan Henry and members of her laboratory, George Carman, Lisa Klig, Shelley Esposito, Camille Steber, Randy Strich, Sepp Kohlwein, Robert Dickson, David Stillman, Mark Johnston, Patricia McGraw, and Terry Cooper for providing information prior to publication and for stimulating discussions.

We are supported by funding from the NIH (M.L.G.) and The Potts Foundation (J.M.L.).

REFERENCES

1. **Al-Feel, W., S. S. Chirala, and S. J. Wakil.** 1992. Cloning of the yeast *FAS3* gene and primary structure of yeast acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. USA **89:**4534–4538.
2. **Allen, T. M., P. Williamson, and R. A. Schlegel.** 1988. Phosphatidylserine as a determinant of reticuloendothelial recognition of liposome models of the erythrocyte surface. Proc. Natl. Acad. Sci. USA **85:**8067–8071.
3. **Amati, B., and H. Land.** 1994. Myc-Max-Mad: a transcription factor network controlling cell cycle progression, differentiation and death. Curr. Opin. Genes Dev. **4:**102–108.
4. **Ambroziak, J., and S. A. Henry.** 1994. *INO2* and *INO4* gene products, positive regulators of phospholipid biosynthesis in *Saccharomyces cerevisiae*, form a complex that binds to the *INO1* promoter. J. Biol. Chem. **269:** 15344–15349.
5. **Arndt, K. M., S. Ricupero-Hovasse, and F. Winston.** 1995. TBP mutants defective in activated transcription *in vivo*. EMBO J. **14:**1490–1497.
6. **Ashburner, B. P., and J. M. Lopes.** 1995. Autoregulated expression of the yeast *INO2* and *INO4* helix-loop-helix activator genes effect cooperative regulation on their target genes. Mol. Cell. Biol. **15:**1709–1715.
7. **Ashburner, B. P., and J. M. Lopes.** 1995. Regulation of yeast phospholipid biosynthetic gene expression in response to inositol involves two superimposed mechanisms. Proc. Natl. Acad. Sci. USA **92:**9722–9726.
8. **Atkinson, K., S. Fogel, and S. A. Henry.** 1980. Yeast mutant defective in phosphatidylserine synthesis. J. Biol. Chem. **255:**6653–6661.
9. **Atkinson, K. D., B. Jensen, A. I. Kolat, E. M. Storm, S. A. Henry, and S. Fogel.** 1980. Yeast mutants auxotrophic for choline or ethanolamine. J. Bacteriol. **141:**558–564.
10. **Auger, K. R., C. L. Carpenter, L. C. Cantley, and L. Varticovski.** 1989. Phosphatidylinositol 3-kinase and its novel product, phosphatidylinositol 3-phosphate, are present in *Saccharomyces cerevisiae*. J. Biol. Chem. **264:** 20181–20184.
11. **Bachhawat, N., Q. Ouyang, and S. A. Henry.** 1995. Functional characterization of the inositol-sensitive upstream activation sequence of yeast. J. Biol. Chem. **270:**25087–25095.
12. **Bailis, A. M., J. M. Lopes, S. D. Kohlwein, and S. A. Henry.** 1992. *cis* and *trans* regulatory elements required for regulation of the *CHO1* gene of *Saccharomyces cerevisiae*. Nucleic Acids Res. **20:**1411–1418.
13. **Bailis, A. M., M. A. Poole, G. M. Carman, and S. A. Henry.** 1987. The membrane-associated enzyme phosphatidylserine synthase is regulated at the level of mRNA abundance. Mol. Cell. Biol. **7:**167–176.
14. **Blackwell, T. K., and H. Weintraub.** 1990. Differences and similarities in DNA-binding preferences of MyoD and E2A protein complexes revealed by binding site selection. Science **250:**1104–1110.
15. **Block, L. H., M. Knorr, E. Vogt, R. Locher, W. Vetter, P. Groscurth, B.-Y. Qiao, D. Pometta, R. James, M. Regenass, and A. Pletscher.** 1988. Low density lipoprotein causes general cellular activation and increased phosphatidylinositol turnover and lipoprotein catabolism. Proc. Natl. Acad. Sci. USA **85:**885–889.
16. **Brent, R.** 1985. Repression of transcription in yeast. Cell **42:**3–4.
17. **Buchman, A. R., and R. D. Kornberg.** 1990. A yeast *ARS*-binding protein activates transcription synergistically in combination with other weak activating factors. Mol. Cell. Biol. **10:**887–897.
18. **Buckingham, L. E., H.-T. Wang, R. T. Elder, R. M. McCarroll, M. R. Slater, and R. E. Esposito.** 1990. Nucleotide sequence and promoter analysis of *SPO13*, a meiosis-specific gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **87:**9406–9410.
19. **Buede, R., C. Rinker-Schaffer, W. J. Pinto, R. L. Lester, and R. C. Dickson.** 1991. Cloning and characterization of *LCB1*, a *Saccharomyces* gene required for biosynthesis of the long-chain base component of sphingolipids. J. Bacteriol. **173:**4325–4332.
20. **Carman, G. M., and S. A. Henry.** 1989. Phospholipid biosynthesis in yeast. Annu. Rev. Biochem. **58:**635–669.
21. **Carson, M. A., M. Emala, P. Hogsten, and C. J. Waechter.** 1984. Coordinate regulation of phosphatidylserine decarboxylase activity and phospholipid *N*-methylation in yeast. J. Biol. Chem. **259:**6267–6273.
22. **Chirala, S. S.** 1992. Coordinated regulation and inositol-mediated and fatty acid-mediated repression of fatty acid synthase genes in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:**10232–10236.
23. **Chirala, S. S., M. A. Kuziora, D. M. Spector, and S. J. Wakil.** 1987. Complementation of mutations and nucleotide sequence of *FAS1* gene encoding β subunit of yeast fatty acid synthase. J. Biol. Chem. **262:**4231–4240.
24. **Chirala, S. S., Q. Zong, W. Huang, and W. Al-Feel.** 1994. Analysis of *FAS3*/*ACC* regulatory region of *Saccharomyces cerevisiae*: identification of a functional UAS_{INO} and sequences responsible for fatty acid mediated repression. Nucleic Acids Res. **22:**412–418.
25. **Clancey, C. J., S.-C. Chang, and W. Dowhan.** 1993. Cloning of a gene (*PSD1*) encoding phosphatidylserine decarboxylase from *Saccharomyces cerevisiae* by complementation of an *Escherichia coli* mutant. J. Biol. Chem. **268:**24580–24590.
26. **Cobon, G. S., P. D. Crowfoot, and A. W. Linnane.** 1974. Biogenesis of mitochondria: phospholipid synthesis *in vitro* by yeast mitochondrial and microsomal fractions. Biochem. J. **144:**265–275.
27. **Culbertson, M. R., T. F. Donahue, and S. A. Henry.** 1976. Control of inositol biosynthesis in *Saccharomyces cerevisiae*: properties of a repressible enzyme system in extracts of wild-type (*Ino*$^+$) cells. J. Bacteriol. **126:**232–242.
28. **Culbertson, M. R., T. F. Donahue, and S. A. Henry.** 1976. Control of inositol biosynthesis in *Saccharomyces cerevisiae*: inositol-phosphate synthetase mutants. J. Bacteriol. **126:**243–250.

29. **Culbertson, M. R., and S. A. Henry.** 1975. Inositol-requiring mutants of *Saccharomyces cerevisiae*. Genetics **80:**23–40.
30. **Daum, G.** 1985. Lipids of mitochondria. Biochim. Biophys. Acta **822:**1–42.
31. **Dean-Johnson, M., and S. A. Henry.** 1989. Biosynthesis of inositol in yeast. J. Biol. Chem. **264:**1274–1283.
32. **Divecha, N., and R. F. Irvine.** 1995. Phospholipid signaling. Cell **80:**269–278.
33. **Donahue, T. F., and S. A. Henry.** 1981. Inositol mutants of *Saccharomyces cerevisiae*: mapping the *ino1* locus and characterizing alleles of the *ino1*, *ino2* and *ino4* loci. Genetics **98:**491–503.
34. **Donahue, T. F., and S. A. Henry.** 1981. *myo*-inositol-1-phosphate synthase. Characteristics of the enzyme and identification of its structural gene in yeast. J. Biol. Chem. **256:**7077–7085.
35. **Eilers, M., T. Endo, and G. Schatz.** 1989. Adriamycin, a drug interacting with acidic phospholipids, blocks import of precursor proteins by isolated yeast mitochondria. J. Biol. Chem. **264:**2945–2950.
36. **Endo, T., M. Eilers, and G. Schatz.** 1989. Binding of a tightly folded artificial mitochondrial precursor protein to the mitochondrial outer membrane involves a lipid-mediated conformational change. J. Biol. Chem. **264:** 2951–2956.
37. **Fernandez, S., M. J. Homann, S. A. Henry, and G. M. Carman.** 1986. Metabolism of the phospholipid precursor inositol and its relationship to growth and viability in the natural auxotroph *Schizosaccharomyces pombe*. J. Bacteriol. **166:**779–786.
38. **Fischl, A. S., and G. M. Carman.** 1983. Phosphatidylinositol biosynthesis in *Saccharomyces cerevisiae*: purification and properties of microsome-associated phosphatidylinositol synthase. J. Bacteriol. **154:**304–311.
39. **Fischl, A. S., M. J. Homann, M. A. Poole, and G. M. Carman.** 1986. Phosphatidylinositol synthase from *Saccharomyces cerevisiae*. J. Biol. Chem. **261:**3178–3183.
40. **Flick, J. S., and M. Johnston.** 1990. Two systems of glucose repression of the *GAL1* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**4757–4769.
41. **Gansheroff, L. J., C. Dollard, P. Tan, and F. Winston.** 1995. The *Saccharomyces cerevisiae SPT7* gene encodes a very acidic protein important for transcription *in vivo*. Genetics **139:**523–536.
42. **Gaynor, P. M., T. Gill, S. Toutenhoofd, E. F. Summers, P. McGraw, M. J. Homann, S. A. Henry, and G. M. Carman.** 1991. Regulation of phosphatidylethanolamine methyltransferase and phospholipid methyltransferase by phospholipid precursors in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1090:**326–332.
43. **Gaynor, P. M., and M. L. Greenberg.** 1992. Regulation of CDP-diacylglycerol synthesis and utilization by inositol and choline in *Schizosaccharomyces pombe*. J. Bacteriol. **174:**5711–5718.
44. **Gaynor, P. M., S. Hubbell, A. J. Schmidt, R. A. Lina, S. A. Minskoff, and M. L. Greenberg.** 1991. Regulation of phosphatidylglycerolphosphate synthase in *Saccharomyces cerevisiae* by factors affecting mitochondrial development. J. Bacteriol. **173:**6124–6131.
45. **Gerber, H.-P., K. Seipel, O. Georgiev, M. Höfferer, M. Hug, S. Rusconi, and W. Schaffner.** 1994. Transcriptional activation modulated by homopolymeric glutamine and proline stretches. Science **263:**808–811.
46. **Goldfine, H., and P. O. Hagen.** 1972. Bacterial plasmalogens, p. 330–350. *In* F. Snyder (ed.), Ether lipids, chemistry and biology. Academic Press, Inc., New York.
47. **Greenberg, M. L.** 1980. Genetic regulation of the biosynthesis and utilization of inositol in yeast. Ph.D. thesis. Albert Einstein College of Medicine, New York.
48. **Greenberg, M. L., P. Goldwasser, and S. A. Henry.** 1982. Characterization of a yeast regulatory mutant constitutive for synthesis of inositol-1-phosphate synthase. Mol. Gen. Genet. **186:**157–163.
49. **Greenberg, M. L., S. Hubbell, and C. Lam.** 1988. Inositol regulates phosphatidylglycerolphosphate synthase expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**4773–4779.
50. **Greenberg, M. L., L. S. Klig, V. A. Letts, B. S. Loewy, and S. A. Henry.** 1983. Yeast mutant defective in phosphatidylcholine synthesis. J. Bacteriol. **153:** 791–799.
51. **Greenberg, M. L., B. Reiner, and S. A. Henry.** 1982. Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol-excreting mutants. Genetics **100:**19–33.
52. **Griggs, D. W., and M. Johnston.** 1991. Regulated expression of the *GAL4* activator gene in yeast provides a sensitive genetic switch for glucose repression. Proc. Natl. Acad. Sci. USA **88:**8597–8601.
53. **Guarente, L.** 1987. Regulatory proteins in yeast. Annu. Rev. Genet. **21:** 425–452.
54. **Guarente, L.** 1988. UASs and enhancers: common mechanism of transcriptional activation in yeast and mammals. Cell **52:**303–305.
55. **Guarente, L., R. R. Yocum, and P. Gifford.** 1982. A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. Proc. Natl. Acad. Sci. USA **79:**7410–7414.
56. **Guinea, R., and L. Carrasco.** 1990. Phospholipid biosynthesis and poliovirus genome replication, two coupled phenomena. EMBO J. **9:**2011–2016.
57. **Hajra, A. K.** 1983. Biosynthesis of O-alkylglycerol ether lipids, p. 85–106. *In* H. K. Mangold and F. Paltauf (ed.), Ether lipids: biochemical and biomedical aspects. Academic Press, Inc., New York.
58. **Hammond, C. I., P. Romano, S. Roe, and P. Tontonoz.** 1993. *INO2*, a regulatory gene in yeast phospholipid biosynthesis, affects nuclear segregation and bud pattern formation. Cell. Mol. Biol. Res. **39:**561–577.
59. **Hasslacher, M., A. S. Ivessa, F. Paltauf, and S. D. Kohlwein.** 1993. Acetyl-CoA carboxylase from yeast is an essential enzyme and is regulated by factors that control phospholipid metabolism. J. Biol. Chem. **268:**1–7.
60. **Henry, S. A., K. D. Atkinson, A. I. Kolat, and M. R. Culbertson.** 1977. Growth and metabolism of inositol-starved *Saccharomyces cerevisiae*. J. Bacteriol. **130:**472–484.
61. **Henry, S. A., T. F. Donahue, and M. R. Culbertson.** 1975. Selection of spontaneous mutants by inositol starvation in yeast. Mol. Gen. Genet. **143:** 5–11.
62. **Hikiji, T., K. Miura, K. Kiyono, I. Shibuya, and A. Ohta.** 1988. Disruption of the *CHO1* gene encoding phosphatidylserine synthase in *Saccharomyces cerevisiae*. J. Biochem. **104:**894–900.
63. **Hill, J. E., C. Chung, P. McGraw, E. Summers, and S. A. Henry.** 1984. Biosynthesis and role of phospholipids in yeast membranes, p. 246–260. *In* P. J. Kuhn, A. P. J. Trinci, M. J. Jung, M. W. Goosey, and L. G. Copping (ed.), Biochemistry of cell walls and membranes of fungi. Springer-Verlag, New York.
64. **Hinnebusch, A. G.** 1984. Evidence for translational regulation of the activator of general amino acid control in yeast. Proc. Natl. Acad. Sci. USA **81:** 6442–6446.
65. **Hirsch, J. P.** 1987. *cis*- and *trans*-acting regulation of the *INO1* gene of *Saccharomyces cerevisiae*. Ph.D. thesis. Albert Einstein College of Medicine, New York.
66. **Hirsch, J. P., and S. A. Henry.** 1986. Expression of the *Saccharomyces cerevisiae* inositol-1-phosphate synthase (*INO1*) gene is regulated by factors that affect phospholipid synthesis. Mol. Cell. Biol. **6:**3320–3328.
67. **Hirschberg, C. B., and E. P. Kennedy.** 1972. Mechanism of the enzymatic synthesis of cardiolipin in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **69:** 648–651.
68. **Hjelmsted, R. H., and R. M. Bell.** 1987. Mutants of Saccharomyces cerevisiae defective in *sn*-1,2-diacylglycerol cholinephosphotransferase. J. Biol. Chem. **262:**3909–3917.
69. **Hjelmsted, R. H., and R. M. Bell.** 1988. The *sn*-1,2-diacylglycerol ethanolaminephosphotransferase activity of *Saccharomyces cerevisiae*. J. Biol. Chem. **263:**19748–19757.
70. **Hjelmsted, R. H., and R. M. Bell.** 1990. The *sn*-1,2-diacylglycerol cholinephosphotransferase of *Saccharomyces cerevisiae*. J. Biol. Chem. **265:**1755–1764.
71. **Holliday, R.** 1962. Selection of auxotrophs by inositol starvation in *Ustilago maydis*. Microbiol. Gen. Bull. **13:**28–30.
72. **Homann, M. J., A. M. Bailis, S. A. Henry, and G. M. Carman.** 1987. Coordinate regulation of phospholipid biosynthesis by serine in *Saccharomyces cerevisiae*. J. Bacteriol. **169:**3276–3280.
73. **Homann, M. J., S. A. Henry, and G. M. Carman.** 1985. Regulation of CDP-diacylglycerol synthase activity in *Saccharomyces cerevisiae*. J. Bacteriol. **163:**1265–1266.
74. **Homann, M. J., M. A. Poole, P. M. Gaynor, C.-T. Ho, and G. M. Carman.** 1987. Effect of growth phase on phospholipid biosynthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **169:**533–539.
75. **Hosaka, K., T. Kodaki, and S. Yamashita.** 1989. Cloning and characterization of the yeast *CKI* gene encoding choline kinase and its expression in *Escherichia coli*. J. Biol. Chem. **264:**2053–2059.
76. **Hosaka, K., T. Murakami, T. Kodaki, J.-I. Nikawa, and S. Yamashita.** 1990. Repression of choline kinase by inositol and choline in *Saccharomyces cerevisiae*. J. Bacteriol. **173:**2005–2012.
77. **Hosaka, K., J.-I. Nikawa, T. Kodaki, and S. Yamashita.** 1992. A dominant mutation that alters the regulation of *INO1* expression in *Saccharomyces cerevisiae*. J. Biochem. **111:**352–358.
78. **Hosaka, K., J.-I. Nikawa, T. Kodaki, and S. Yamashita.** 1994. Cloning and characterization of the *SCS1* gene required for the expression of genes in yeast phospholipid synthesis. J. Biochem. **115:**131–136.
79. **Hosaka, K., and S. Yamashita.** 1980. Choline transport in *Saccharomyces cerevisiae*. J. Bacteriol. **143:**176–181.
80. **Hoshizaki, D. K., J. E. Hill, and S. A. Henry.** 1990. The *Saccharomyces cerevisiae INO4* gene encodes a small, highly basic protein required for derepression of phospholipid biosynthetic enzymes. J. Biol. Chem. **265:** 4736–4745.
81. **Hromy, J. M., and G. M. Carman.** 1986. Reconstitution of Saccharomyces cerevisiae phosphatidylserine synthase into phospholipid vesicles. J. Biol. Chem. **261:**15572–15576.
82. **Hubbard, S. C., and S. Brody.** 1975. Glycerophospholipid variation in choline and inositol auxotrophs of *Neurospora crassa*. J. Biol. Chem. **250:** 7173–7181.
83. **Hudak, K. A., J. M. Lopes, and S. A. Henry.** 1994. A pleiotropic phospholipid biosynthetic regulatory mutation in *Saccharomyces cerevisiae* is allelic to *sin3* (*sdi1*, *ume4*, *rpd1*). Genetics **136:**475–483.
84. **Jackson, J. C., and J. M. Lopes.** The yeast *UME6* gene is required for both negative and positive transcriptional regulation of phospholipid biosyn-

thetic gene expression. Submitted for publication.
85. **Jackson, M., and S. Shin.** 1980. Analysis of inositol starvation in an inositol requiring mutant CHO-K1. J. Cell Biol. **87:**293a.
86. **Jakovcic, S. G., S. Getz, M. Rabinowitz, H. Jacob, and H. Swift.** 1971. Cardiolipin content of wild type and mutant yeasts in relation to mitochondrial function and development. J. Cell Biol. **40:**490–502.
87. **Jayaraman, P.-S., K. Hirst, and C. R. Goding.** 1994. The activation domain of a basic helix-loop-helix protein is masked by repressor interaction with domains distinct from that required for transcription regulation. EMBO J. **13:**2192–2199.
88. **Johnston, M.** 1987. A model fungal gene regulatory mechanism: the *GAL* genes of *Saccharomyces cerevisiae*. Microbiol. Rev. **51:**458–476.
89. **Johnston, M. R., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:**1440–1449.
90. **Kadenbach, B., P. Mende, H. V. Kolbe, I. Stipani, and F. Palmieri.** 1982. The mitochondrial phosphate carrier has an essential requirement for cardiolipin. FEBS Lett. **139:**109–112.
90a. **Kanipes, M. I., and S. A. Henry.** Personal communication.
91. **Karkhoff-Schweizer, R. R., B. L. Kelly, and M. L. Greenberg.** 1991. Phosphatidylglycerol synthase expression in *Schizosaccharomyces pombe* is regulated by the phospholipid precursors inositol and choline. J. Bacteriol. **173:** 6132–6138.
92. **Kelley, M. J., A. M. Bailis, S. A. Henry, and G. M. Carman.** 1988. Regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae* by inositol. J. Biol. Chem. **263:**18078–18085.
93. **Kelley, M. J., and G. M. Carman.** 1987. Purification and characterization of CDP-diacylglycerol synthase from *Saccharomyces cerevisiae*. J. Biol. Chem. **262:**14563–14570.
94. **Kelly, B. L., and M. L. Greenberg.** 1990. Characterization and regulation of phosphatidylglycerolphosphate phosphatase in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1046:**144–150.
95. **Kennedy, E. P., and S. B. Weiss.** 1956. The function of cytidine coenzymes in the biosynthesis of phospholipids. J. Biol. Chem. **222:**193–214.
96. **Kim, Y.-J., S. Bjorklund, Y. Li, M. H. Sayre, and R. D. Kornberg.** 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:**599–608.
97. **Kinney, A. J., M. Bae-Lee, S. S. Panghaal, M. J. Kelley, P. M. Gaynor, and G. M. Carman.** 1990. Regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae* by cyclic AMP-dependent protein kinase. J. Bacteriol. **172:**1133–1136.
98. **Kinney, A. J., and G. M. Carman.** 1988. Phosphorylation of yeast phosphatidylserine synthase *in vivo* and *in vitro* by cyclic AMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA **85:**7962–7966.
99. **Kiyono, K., K. Miura, Y. Kushima, T. Hikiji, J. Fukushima, I. Shibuya, and A. Ohta.** 1987. Primary structure and product characterization of the *Saccharomyces cerevisiae CHO1* gene that encodes phosphatidylserine synthase. J. Biochem. **102:**1089–1100.
100. **Klig, L. S., B. Antonsson, E. Schmid, and L. Friedli.** 1991. Inositol biosynthesis: *Candida albicans* and *Saccharomyces cerevisiae* genes share common regulation. Yeast **7:**325–336.
101. **Klig, L. S., L. Friedli, and E. Schmid.** 1990. Phospholipid biosynthesis in *Candida albicans*: regulation by the precursors inositol and choline. J. Bacteriol. **172:**4407–4414.
102. **Klig, L. S., and S. A. Henry.** 1984. Isolation of the yeast *INO1* gene: located on an autonomously replicating plasmid, the gene is fully regulated. Proc. Natl. Acad. Sci. USA **81:**3816–3820.
103. **Klig, L. S., M. J. Homann, G. M. Carman, and S. A. Henry.** 1985. Coordinate regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*: pleiotropically constitutive *opi1* mutant. J. Bacteriol. **162:**1135–1141.
104. **Klig, L. S., M. J. Homann, S. D. Kohlwein, M. J. Kelley, S. A. Henry, and G. M. Carman.** 1988. *Saccharomyces cerevisiae* mutant with a partial defect in the synthesis of CDP-diacylglycerol and altered regulation of phospholipid biosynthesis. J. Bacteriol. **170:**1878–1886.
105. **Klig, L. S., D. K. Hoshizaki, and S. A. Henry.** 1988. Isolation of the yeast *INO4* gene, a positive regulator of phospholipid biosynthesis. Curr. Genet. **13:**7–14.
106. **Klig, L. S., P. A. Zobel, C. G. Devry, and C. Losberger.** 1994. Comparison of *INO1* gene sequences and products in *Candida albicans* and *Saccharomyces cerevisiae*. Yeast **10:**789–800.
107. **Ko, J., S. Cheah, and A. S. Fischl.** 1994. Regulation of phosphatidylinositol: ceramide phosphoinositol transferase in *Saccharomyces cerevisiae*. J. Bacteriol. **176:**5181–5183.
108. **Kodaki, T., K. Hosaka, J.-I. Nikawa, and S. Yamashita.** 1991. Identification of the upstream activation sequences responsible for the expression and regulation of the *PEM1* and *PEM2* genes encoding the enzymes of the phosphatidylethanolamine methylation pathway in *Saccharomyces cerevisiae*. J. Biochem. **109:**276–287.
109. **Kodaki, T., K. Hosaka, J.-I. Nikawa, and S. Yamashita.** 1995. The *SNF2/SWI2/GAM1/TYE3/RIC1* gene is involved in the coordinate regulation of phospholipid synthesis in *Saccharomyces cerevisiae*. J. Biochem. **117:**362–368.
110. **Kodaki, T., J.-I. Nikawa, K. Hosaka, and S. Yamashita.** 1991. Functional analysis of the regulatory region of the yeast phosphatidylserine synthase gene, *PSS*. J. Bacteriol. **173:**7992–7995.
111. **Kodaki, T., and S. Yamashita.** 1987. Yeast phosphatidylethanolamine methylation pathway. J. Biol. Chem. **262:**15428–15435.
112. **Kodaki, T., and S. Yamashita.** 1989. Characterization of the methyltransferases in the yeast phosphatidylethanolamine methylation pathway by selective gene disruption. Eur. J. Biochem. **185:**243–251.
113. **Koleske, A. J., S. Buratowski, M. Nonet, and R. A. Young.** 1992. A novel transcription factor reveals a functional link between the RNA polymerase II CTD and TFIID. Cell **69:**883–894.
114. **Koleske, A. J., and R. A. Young.** 1994. An RNA polymerase II holoenzyme responsive to activators. Nature (London) **368:**466–469.
115. **Kornitzer, D., B. Ralboy, R. G. Kulka, and G. R. Fink.** 1994. Regulated degradation of the transcription factor Gcn4. EMBO J. **13:**6021–6039.
116. **Kovác, L., I. Gbleská, V. Poliachová, J. Subík, and V. Kovácová.** 1980. Membrane mutants: a yeast mutant with a lesion in phosphatidylserine biosynthesis. Eur. J. Biochem. **111:**491–501.
117. **Kuchler, K., G. Daum, and F. Paltauf.** 1986. Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. J. Bacteriol. **165:**901–910.
118. **Kuhl, D. P. A., and C. T. Caskey.** 1993. Trinucleotide repeats and genome variation. Curr. Opin. Genes Dev. **3:**404–407.
119. **Kumano, Y., and J.-I. Nikawa.** 1995. Functional analysis of mutations in the PIS gene, which encodes Saccharomyces cerevisiae phosphatidylinositol synthase. FEMS Microbiol. Lett. **126:**81–84.
120. **Kuo, M.-H., and E. Grayhack.** 1994. A library of yeast genomic MCM1 binding sites contains genes involved in cell cycle control, cell wall and membrane structure, and metabolism. Mol. Cell. Biol. **14:**348–359.
121. **Lai, K., C. P. Bolognese, S. Swift, and P. McGraw.** 1995. Regulation of inositol transport in *Saccharomyces cerevisiae* involves inositol-induced changes in permease stability and endocytic degradation in the vacuole. J. Biol. Chem. **270:**2525–2534.
122. **Lai, K., and P. McGraw.** 1994. Dual control of inositol transport in *Saccharomyces cerevisiae* by irreversible inactivation of permease and regulation of permease synthesis by *INO2*, *INO4*, and *OPI1*. J. Biol. Chem. **269:** 2245–2251.
123. **Lamphier, M. S., and M. Ptashne.** 1992. Multiple mechanisms mediate glucose repression of the yeast *GAL1* gene. Proc. Natl. Acad. Sci. USA **89:** 5922–5926.
124. **Lamping, E., J. Lückl, F. Paltauf, S. A. Henry, and S. D. Kohlwein.** 1995. Isolation and characterization of a mutant of *Saccharomyces cerevisiae* with pleiotropic deficiencies in transcriptional activation and repression. Genetics **137:**55–65.
125. **Lamping, E. L., S. D. Kohlwein, S. A. Henry, and F. Paltauf.** 1991. Coordinate regulation of phosphatidylserine decarboxylase in *Saccharomyces cerevisiae*. J. Bacteriol. **173:**6432–6437.
126. **Landschulz, W. H., P. F. Johnson, and S. L. McKnight.** 1988. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science **240:**1759–1764.
127. **Landschulz, W. H., P. F. Johnson, and S. L. McKnight.** 1989. The DNA binding domain of the rat liver nuclear protein C/EBP is bipartite. Science **243:**1681–1688.
128. **Le Petit-Thevenin, J., B. Lerique, J. O. Nobili, and J. Boyer.** 1991. Estrogen modulates phospholipid acylation in red blood cells: relationship to cell aging. Am. J. Physiol. **261:**C423–C427.
129. **Le Petit-Thevenin, J., J. O. Nobili, and J. Boyer.** 1991. Changes in pattern of phospholipid acylation during in vivo aging of rat blood cells. Am. J. Physiol. **261:**C417–C422.
130. **Lester, R. L., S. Smith, G. Wells, D. Rees, and W. Angus.** 1974. The isolation and partial characterization of two novel sphingolipids from *Neurospora crassa*: di(inositol-phosphoryl)ceramide and $[(gal)_3glu]$ceramide. J. Biol. Chem. **249:**3388–3394.
131. **Lester, R. L., and M. R. Steiner.** 1968. The occurrence of diphosphoinositide and triphosphoinositide in *Saccharomyces cerevisiae*. J. Biol. Chem. **243:**4889–4893.
132. **Letts, V. A., L. S. Klig, M. Bae-Lee, G. M. Carman, and S. A. Henry.** 1983. Isolation of the yeast structural gene for the membrane-associated enzyme phosphatidylserine synthase. Proc. Natl. Acad. Sci. USA **80:**7279–7283.
133. **Leuther, K. K., and S. A. Johnston.** 1992. Nondissociation of GAL4 and GAL80 *in vivo* after galactose induction. Science **256:**1333–1335.
134. **Levine, M., and J. L. Manley.** 1989. Transcriptional repression of eukaryotic promoters. Cell **59:**405–408.
135. **Li, Y., and R. D. Kornberg.** 1994. Interplay of positive and negative effectors in function of the C-terminal repeat domain of RNA polymerase II. Proc. Natl. Acad. Sci. USA **91:**2362–2366.
136. **Li, Z., and M. Brendel.** 1993. Co-regulation with genes of phospholipid biosynthesis of the *CTR/HNM1*-encoded choline/nitrogen mustard permease in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **241:**680–684.
137. **Li, Z., E. Haase, and M. Brendel.** 1991. Hyper-resistance to nitrogen mustard in *Saccharomyces cerevisiae* is caused by a defective choline transport. Curr. Genet. **19:**423–427.

138. **Lloyd-Still, J. D., S. B. Johnson, and R. Holman.** 1991. Essential fatty acid status and fluidity of plasma phospholipids in cystic fibrosis infants. Am. J. Clin. Nutr. **54:**1029–1035.
139. **Loewy, B. S., and S. A. Henry.** 1984. The *INO2* and *INO4* loci of *Saccharomyces cerevisiae* are pleiotropic regulatory genes. Mol. Cell. Biol. **4:**2479–2485.
140. **Lopes, J. M., and S. A. Henry.** 1991. Interaction of *trans* and *cis* regulatory elements in the *INO1* promoter of *Saccharomyces cerevisiae*. Nucleic Acids Res. **19:**3987–3994.
141. **Lopes, J. M., J. P. Hirsch, P. A. Chorgo, K. L. Schulze, and S. A. Henry.** 1991. Analysis of sequences in the *INO1* promoter that are involved in its regulation by phospholipid precursors. Nucleic Acids Res. **19:**1687–1693.
142. **Lopes, J. M., K. L. Schulze, J. W. Yates, J. P. Hirsch, and S. A. Henry.** 1993. The *INO1* promoter of *Saccharomyces cerevisiae* includes an upstream repressor sequence (URS1) common to a diverse set of yeast genes. J. Bacteriol. **175:**4235–4238.
143. **Luche, R. M., W. C. Smart, and T. G. Cooper.** 1992. Purification of the heteromeric protein binding to the URS1 transcriptional repression site in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:**7412–7416.
144. **Luche, R. M., R. Sumrada, and T. G. Cooper.** 1990. A *cis*-acting element present in multiple genes serves as a repressor protein-binding site for the yeast *CAR1* gene. Mol. Cell. Biol. **10:**3884–3895.
145. **Lynn, W. S., A. Tweedale, and M. W. Cloyd.** 1988. Human immunodeficiency virus (HIV-1) cytotoxicity: perturbation of the cell membrane and derepression of phospholipid synthesis. Virology **163:**43–51.
146. **Mangold, H. K.** 1972. The search for alkoxylipids in plants, p. 399–405. *In* F. Snyder (ed.), Ether lipids, chemistry and biology. Academic Press, Inc., New York.
147. **Matsumoto, K., I. Uno, and T. Ishikawa.** 1983. Initiation of meiosis in yeast mutants defective in adenylate cyclase and cyclic AMP-dependent protein kinase. Cell **32:**417–423.
148. **Matsumoto, K., I. Uno, Y. Oshima, and T. Ishikawa.** 1982. Isolation and characterization of yeast mutants deficient in adenylate cyclase and cyclic AMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA **79:**2355–2359.
149. **Matsushita, M., and J. I. Nikawa.** 1995. Isolation and characterization of a *SCT1* gene which can suppress a choline-transport mutant of *Saccharomyces cerevisiae*. J. Biochem. **117:**447–451.
150. **McGraw, P., and S. A. Henry.** 1989. Mutations in the *Saccharomyces cerevisiae opi3* gene: effects on phospholipid methylation, growth and cross-pathway regulation of inositol synthesis. Genetics **122:**317–330.
151. **McMaster, C. R., and R. M. Bell.** 1994. Phosphatidylcholine biosynthesis via the CDP-choline pathway in *Saccharomyces cerevisiae*. J. Biol. Chem. **269:**14776–14783.
152. **McVeigh, I., and E. Bracken.** 1955. The nutrition of *Schizosaccharomyces pombe*. Mycologia **47:**13–25.
153. **Megnet, R.** 1964. A method for the selection of auxotrophic mutants of the yeast *Schizosaccharomyces pombe*. Experientia **20:**320–321.
154. **Minskoff, S. A., P. M. Gaynor, and M. L. Greenberg.** 1992. Mutant enrichment of *Schizosaccharomyces pombe* by inositol-less death. J. Bacteriol. **174:**4078–4085.
155. **Minskoff, S. A., P. V. Racenis, J. Granger, L. Larkins, A. K. Hajra, and M. L. Greenberg.** 1994. Regulation of phosphatidic acid biosynthetic enzymes in *Saccharomyces cerevisiae*. J. Lipid Res. **35:**2254–2262.
156. **Mohamed, A. H., S. S. Chirala, N. H. Mody, W.-Y. Huang, and S. J. Wakil.** 1988. Primary structure of the multifunctional α subunit protein of yeast fatty acid synthase derived from *FAS2* gene sequence. J. Biol. Chem. **263:**12315–12325.
157. **Moore, T. S.** 1982. Phospholipid biosynthesis. Annu. Rev. Plant Physiol. **33:**235–259.
158. **Murre, C., P. S. McCaw, and D. Baltimore.** 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD*, and *myc* proteins. Cell **56:**777–783.
159. **Nagiec, M. M., J. A. Baltisberger, G. B. Wells, R. L. Lester, and R. C. Dickson.** 1994. The *LCB2* gene of *Saccharomyces* and the related *LCB1* gene encode subunits of serine palmitoyltransferase, the initial enzyme in sphingolipid synthesis. Proc. Natl. Acad. Sci. USA **91:**7899–7902.
160. **Nagiec, M. M., G. B. Wells, R. L. Lester, and R. C. Dickson.** 1993. A suppressor gene that enables *Saccharomyces cerevisiae* to grow without making sphingolipids encodes a protein that resembles an *Escherichia coli* fatty acyltransferase. J. Biol. Chem. **268:**22156–22163.
161. **Nikawa, J.-I., K. Hosaka, Y. Tsukagoshi, and S. Yamashita.** 1990. Primary structure of the yeast choline transport gene and regulation of its expression. J. Biol. Chem. **265:**15996–16003.
162. **Nikawa, J.-I., T. Kodaki, and S. Yamashita.** 1987. Primary structure and disruption of the phosphatidylinositol synthase gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **262:**4876–4881.
163. **Nikawa, J.-I., A. Murakami, E. Esumi, and S. Yamashita.** 1995. Cloning and sequence of the *SCS2* gene, which can suppress the defect of *INO1* expression in an inositol auxotrophic mutant of *Saccharomyces cerevisiae*. J. Biochem. **118:**39–45.
164. **Nikawa, J.-I., T. Nagumo, and S. Yamashita.** 1982. *myo*-inositol transport in *Saccharomyces cerevisiae*. J. Bacteriol. **150:**441–446.
165. **Nikawa, J.-I., Y. Tsukagoshi, T. Kodaki, and S. Yamashita.** 1987. Nucleotide sequence and characterization of the yeast *PSS* gene encoding phosphatidylserine synthase. Eur. J. Biochem. **167:**7–12.
166. **Nikawa, J.-I., Y. Tsukagoshi, and S. Yamashita.** 1986. Cloning of a gene encoding choline transport in *Saccharomyces cerevisiae*. J. Bacteriol. **166:**328–330.
167. **Nikawa, J.-I., Y. Tsukagoshi, and S. Yamashita.** 1991. Isolation and characterization of two distinct *myo*-inositol transporter genes of *Saccharomyces cerevisiae*. J. Biol. Chem. **266:**11184–11191.
168. **Nikawa, J.-I., and S. Yamashita.** 1981. Characterization of phosphatidylserine synthase from *Saccharomyces cerevisiae* and a mutant defective in the enzyme. Biochim. Biophys. Acta **665:**420–426.
169. **Nikawa, J.-I., and S. Yamashita.** 1982. Yeast mutant defective in synthesis of phosphatidylinositol. Eur. J. Biochem. **125:**445–451.
170. **Nikawa, J.-I., and S. Yamashita.** 1983. 2-Hydroxyethylhydrazine as a potent inhibitor of phospholipid methylation in yeast. Biochim. Biophys. Acta **751:**201–209.
171. **Nikawa, J.-I., and S. Yamashita.** 1984. Molecular cloning of the gene encoding CDPdiacylglycerol-inositol 3-phosphatidyl transferase in *Saccharomyces cerevisiae*. Eur. J. Biochem. **143:**251–256.
172. **Nikawa, J.-I., and S. Yamashita.** 1992. *IRE1* encodes a putative protein kinase containing a membrane-spanning domain and is required for inositol prototrophy in *Saccharomyces cerevisiae*. Mol. Microbiol. **6:**1441–1446.
173. **Nikoloff, D. M., and S. A. Henry.** 1991. Genetic analysis of yeast phospholipid biosynthesis. Annu. Rev. Genet. **25:**559–583.
174. **Nikoloff, D. M., and S. A. Henry.** 1994. Functional characterization of the *INO2* gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **269:**7402–7411.
175. **Nikoloff, D. M., P. McGraw, and S. A. Henry.** 1992. The *INO2* gene of *Saccharomyces cerevisiae* encodes a helix-loop-helix protein that is required for activation of phospholipid synthesis. Nucleic Acids Res. **20:**3253.
176. **Nitsch, R. M., J. K. Blusztajn, A. J. Pittas, B. E. Slack, J. H. Growdon, and R. J. Wurtman.** 1992. Evidence for a membrane defect in Alzheimer disease brain. Proc. Natl. Acad. Sci. USA **89:**1671–1675.
177. **Nogi, Y., K. Matsumoto, A. Toh-E, and Y. Oshima.** 1977. Interaction of super-repressible and dominant constitutive mutations for the synthesis of galactose pathway enzymes in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **152:**137–144.
178. **Nonet, M. L., and R. A. Young.** 1989. Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of *Saccharomyces cerevisiae* RNA polymerase II. Genetics **123:**715–724.
179. **Paltauf, F., S. D. Kohlwein, and S. A. Henry.** 1992. Regulation and compartmentalization of lipid synthesis in yeast, p. 415–499. *In* E. W. Jones, J. R. Pringle, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: gene expression, vol. II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
180. **Park, H.-D., R. M. Luche, and T. G. Cooper.** 1992. The yeast *UME6* gene product is required for transcriptional repression mediated by the *CAR1 URS1* repressor binding site. Nucleic Acids Res. **20:**1909–1915.
181. **Parthun, M. R., and J. A. Jaehning.** 1990. Purification and characterization of the yeast transcriptional activator GAL4. J. Biol. Chem. **265:**209–213.
182. **Pascal, E., and R. Tjian.** 1991. Different activation domains of Sp1 govern formation of multimers and mediate transcriptional synergism. Genes Dev. **5:**1646–1656.
183. **Peterson, C. L., A. Dingwall, and M. P. Scott.** 1994. Five *SWI/SNF* gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA **91:**2905–2908.
184. **Peterson, C. L., and I. Herskowitz.** 1992. Characterization of the yeast *SWI1*, *SWI2*, and *SWI3* genes, which encode a global activator of transcription. Cell **68:**573–583.
185. **Peterson, C. L., W. Kruger, and I. Herskowitz.** 1991. A functional interaction between the C-terminal domain of RNA polymerase II and the negative regulator SIN1. Cell **64:**1135–1143.
186. **Poole, M. A., M. J. Homann, M. S. Bae-Lee, and G. M. Carman.** 1986. Regulation of phosphatidylserine synthase from *Saccharomyces cerevisiae* by phospholipid precursors. J. Bacteriol. **168:**668–672.
187. **Racenis, P. V.** 1994. Phosphatidate biosynthesis in the yeast *Saccharomyces cerevisiae*. Ph.D. thesis. University of Michigan, Ann Arbor.
188. **Racenis, P. V., J. L. Lai, A. K. Das, P. C. Mullick, A. K. Hajra, and M. L. Greenberg.** 1992. The acyl dihydroxyacetone phosphate pathway enzymes for glycerolipid biosynthesis are present in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **174:**5702–5710.
189. **Richards, R. I., and G. R. Sutherland.** 1992. Dynamic mutations: a new class of mutations causing human disease. Cell **70:**709–712.
190. **Robinson, N. C.** 1982. Specificity and binding affinity of phospholipids to the high-affinity cardiolipin sites of beef heart cytochrome *c* oxidase. Biochemistry **21:**184–188.
191. **Scafe, C., D. Chao, J. Lopes, J. P. Hirsch, S. Henry, and R. A. Young.** 1990. RNA polymerase II C-terminal repeat influences response to transcriptional enhancer signals. Nature (London) **347:**491–494.
192. **Scafe, C., C. Martin, M. Nonet, S. Podos, S. Okamura, and R. A. Young.** 1990. Conditional mutations occur predominantly in highly conserved res-

idues of RNA polymerase II subunits. Mol. Cell. Biol. **10:**1270–1275.
193. **Scafe, C., M. Nonet, and R. A. Young.** 1990. RNA polymerase II mutants defective in transcription of a subset of genes. Mol. Cell. Biol. **10:**1010–1016.
194. **Schlossman, D. M., and R. M. Bell.** 1977. Glycerolipid biosynthesis in *Saccharomyces cerevisiae*: *sn*-glycerol-3-phosphate and dihydroxyacetone phosphate acyltransferase activities. J. Bacteriol. **133:**1368–1376.
195. **Schüller, H.-J., A. Hahn, F. Tröster, A. Schütz, and E. Schweizer.** 1992. Coordinate genetic control of yeast fatty acid synthase genes *FAS1* and *FAS2* by an upstream activation site common to genes involved in membrane lipid biosynthesis. EMBO J. **11:**107–114.
196. **Schüller, H.-J., R. Schorr, B. Hofmann, and E. Schweizer.** 1992. Regulatory gene *INO4* of yeast phospholipid biosynthesis is positively autoregulated and functions as a *trans*-activator of fatty acid synthase genes *FAS1* and *FAS2* from *Saccharomyces cerevisiae*. Nucleic Acids Res. **20:**5955–5961.
197. **Schwank, S., R. Ebbert, K. Rautenstrauss, E. Schweizer, and H.-J. Schüller.** 1995. Yeast transcriptional activator *INO2* interacts as an Ino2p/Ino4p basic helix-loop-helix heteromeric complex with the inositol/choline-responsive element necessary for expression of phospholipid biosynthetic genes in *Saccharomyces cerevisiae*. Nucleic Acids Res. **23:**230–237.
198. **Seta, F. D., S.-A. Ciafré, C. Marck, B. Santoro, C. Presutti, A. Sentenac, and I. Bozzoni.** 1990. The ABF1 factor is the transcriptional activator of the L2 ribosomal protein genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**2437–2441.
199. **Slekar, K. H., and S. A. Henry.** 1995. *SIN3* works through two different promoter elements to regulate *INO1* gene expression in yeast. Nucleic Acids Res. **23:**1964–1969.
200. **Sprague, G. F., Jr., and J. E. Cronan, Jr.** 1977. Isolation and characterization of *Saccharomyces cerevisiae* mutants defective in glycerol catabolism. J. Bacteriol. **129:**1335–1342.
201. **Steiner, M. R., and R. L. Lester.** 1972. *In vitro* studies of phospholipid biosynthesis in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **260:**222–243.
202. **Sternberg, P. W., M. J. Stern, I. Clark, and I. Herskowitz.** 1987. Activation of the yeast *HO* gene by release from multiple negative controls. Cell **48:**567–577.
203. **Stevens, B.** 1981. Mitochondrial structure, p. 471–504. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
204. **Strauss, B. S.** 1958. Cell death and unbalanced growth in *Neurospora*. J. Gen. Microbiol. **18:**658–669.
205. **Strich, R., M. R. Slater, and R. E. Esposito.** 1989. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA **86:**10018–10022.
206. **Strich, R., R. T. Surosky, C. Steber, E. Dubois, F. Messenguy, and R. E. Esposito.** 1994. *UME6* is a key regulator of nitrogen repression and meiotic development. Genes Dev. **8:**796–810.
207. **Struhl, K.** 1989. Molecular mechanisms of transcriptional regulation in yeast. Annu. Rev. Biochem. **58:**1051–1077.
208. **Summers, E. F., V. A. Letts, P. McGraw, and S. A. Henry.** 1988. *Saccharomyces cerevisiae cho2* mutants are deficient in phospholipid methylation and cross-pathway regulation of inositol synthesis. Genetics **120:**909–922.
209. **Swift, S., and P. McGraw.** 1995. *INO1-100*: an allele of the *Saccharomyces cerevisiae INO1* gene that is transcribed without the action of the positive factors encoded by the *INO2*, *INO4*, *SWI1*, *SWI2* and *SWI3* genes. Nucleic Acids Res. **23:**1426–1433.
210. **Tamai, K. T., and M. L. Greenberg.** 1990. Biochemical characterization and regulation of cardiolipin synthase in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1046:**215–222.
211. **Thomas, P. L.** 1972. Increased frequency of auxotrophic mutants of *Ustilago hordei* after combined UV irradiation and inositol starvation. Can. J. Genet. Cytol. **14:**785–788.
212. **Tillman, T. S., and R. M. Bell.** 1986. Mutants of *Saccharomyces cerevisiae* defective in *sn*-glycerol-3-phosphate acyltransferase. J. Biol. Chem. **261:**9144–9149.
213. **Trivedi, A., M. Schwab, D. Fantin, and E. R. Tustanoff.** 1987. Relationship of cardiolipin to cytochrome *c* oxidase kinetics as probed by adriamycin, p. 319–328. *In* K. W. A. Wirtz (ed.), Membrane receptors, dynamics and energetics. Plenum Publishing Corp., New York.
214. **Trivedi, A., A. V. Wearring, S. D. Kohlwein, F. Paultaf, and E. R. Tustanoff.** 1988. Functional importance of mitochondrial cardiolipin in yeast cytochrome *c* oxidase activity, p. 87–94. *In* J. J. Lemasters, C. R. Hackenbrock, R. G. Thurman, and H. V. Weterhoff (ed.), Integration of mitochondrial function. Plenum Publishing Corp., New York.
215. **Trotter, P. J., J. Pedretti, and D. R. Voelker.** 1993. Phosphatidylserine decarboxylase from *Saccharomyces cerevisiae*. J. Biol. Chem. **268:**21416–21424.
216. **Trotter, P. J., J. Pedretti, R. Yates, and D. R. Voelker.** 1995. Phosphatidylserine decarboxylase 2 of *Saccharomyces cerevisiae*. J. Biol. Chem. **270:**6071–6080.
217. **Trotter, P. J., and D. R. Voelker.** 1995. Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (PSD2) in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **270:**6062–6070.
218. **Tsukagoshi, Y., J.-I. Nikawa, K. Hosaka, and S. Yamashita.** 1991. Expression in *Escherichia coli* of the *Saccharomyces cerevisiae CCT* gene encoding cholinephosphate cytidylyltransferase. J. Bacteriol. **173:**2134–2136.
219. **Vidal, M., R. Strich, R. E. Esposito, and R. F. Gaber.** 1991. *RPD1* (*SIN3*/*UME4*) is required for maximal activation and repression of diverse yeast genes. Mol. Cell. Biol. **11:**6306–6316.
220. **Wang, H., I. Clark, P. R. Nicholson, I. Herskowitz, and D. J. Stillman.** 1990. The *Saccharomyces cerevisiae SIN3* gene, a negative regulator of *HO*, contains four paired amphipathic helix motifs. Mol. Cell. Biol. **10:**5927–5936.
221. **Wang, H., and D. J. Stillman.** 1990. *In vitro* regulation of a *SIN3*-dependent DNA-binding by stimulatory and inhibitory factors. Proc. Natl. Acad. Sci. USA **87:**9761–9765.
222. **Wang, H., and D. J. Stillman.** 1993. Transcriptional repression in *Saccharomyces cerevisiae* by a SIN3-LexA fusion protein. Mol. Cell. Biol. **13:**1805–1814.
223. **Weintraub, H.** 1993. The *MyoD* family and myogenesis: redundancy, networks, and thresholds. Cell **75:**1241–1244.
224. **White, M. J., J. P. Hirsch, and S. A. Henry.** 1991. The *OPI1* gene of *Saccharomyces cerevisiae*, a negative regulator of phospholipid biosynthesis, encodes a protein containing polyglutamine tracts and a leucine zipper. J. Biol. Chem. **266:**863–872.
225. **White, M. J., J. M. Lopes, and S. A. Henry.** 1991. Inositol metabolism in yeasts. Adv. Microb. Physiol. **32:**2–47.
226. **Wiame, J. M.** 1971. The regulation of arginine metabolism in *Saccharomyces cerevisiae*: exclusion mechanisms. Curr. Top. Cell. Regul. **4:**1–38.
227. **Yamashita, S., A. Oshima, J.-I. Nikawa, and K. Hosaka.** 1982. Regulation of the phosphatidylethanolamine methylation pathway in *Saccharomyces cerevisiae*. Eur. J. Biochem. **128:**589–595.
228. **Yao, Z., and D. E. Vance.** 1989. Head group specificity in the requirement of phosphatidylcholine biosynthesis for very low density lipoprotein secretion from cultured hepatocytes. J. Biol. Chem. **264:**11373–11380.
229. **Yoshimoto, H., M. Ohmae, and I. Yamashita.** 1992. The *Saccharomyces cerevisiae* GAM2/SIN3 protein plays a role in both activation and repression of transcription. Mol. Gen. Genet. **223:**327–330.
230. **Zinser, E., C. D. M. Sperka-Gottlieb, E. V. Fasch, S. D. Kohlwein, F. Paltauf, and G. Daum.** 1991. Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*. J. Bacteriol. **173:**2026–2034.