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The yeast *UME6* gene is required for both negative and positive transcriptional regulation of phospholipid biosynthetic gene expression

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ABSTRACT

In *Saccharomyces cerevisiae*, regulation of the phospholipid biosynthetic genes, *INO1*, *CHO1*, *CHO2* and *OPI3*, is known to occur at the level of transcript abundance. Derepression in response to inositol deprivation requires the *INO2* and *INO4* regulatory genes. Repression in response to inositol supplementation requires the *OPI1* regulatory gene. Here, we examined the role of the *UME6* global negative regulatory gene in expression of the phospholipid biosynthetic genes. These studies were stimulated by the finding that the *INO1* promoter included a *UME6* cognate *cis*-acting regulatory sequence (URS1). We found that the *UME6* negative regulatory gene was involved in regulation of phospholipid biosynthetic gene expression through two distinct regulatory pathways. One pathway was the direct repression of *INO1* expression through the URS1 element. Surprisingly, the *UME6* gene was also required for derepression of *CHO1*, *CHO2* and *OPI3* gene expression. Consistent with this observation, the *UME6* gene was required for wild-type levels of expression of the *INO2* positive regulatory gene. Therefore, the *UME6* gene has both a negative and a positive role in regulating phospholipid biosynthesis.

INTRODUCTION

In yeast, transcription is carried out by RNA polymerase II in concert with a set of general transcription factors including TFIID, TFIIA and TFIIB (1). Transcription is regulated through the action of gene-specific transcription factors which bind in a sequence-specific manner to regulatory regions found in promoters (1). While considerable progress has been made in understanding the mechanisms controlling the activation of gene expression, transcriptional repression is also important in the regulation of many genes (2). The yeast *UME6* gene product represses transcription of a diverse set of genes involved in meiosis (3–5), heat shock response (6) and arginine catabolism (7). The *UME6* gene is also a positive regulator of some early meiotic genes in sporulating cells (8,9). The molecular mechanism of how the *UME6* gene product functions as an activator is currently unknown. However, the transcriptional activation function is known to be dependent on the *IME1* gene and has only been observed in sporulating cells (8,9). Here, we examined the role of the *UME6* gene in expression of the yeast phospholipid biosynthetic genes under vegetative growth conditions.

In *Saccharomyces cerevisiae*, regulation of the genes in the phospholipid biosynthetic pathway in response to the soluble lipid precursors inositol and choline has been shown to occur at the level of transcription of the *INO1* (10), *CHO1* (11), *CHO2* and *OPI3* structural genes (12,13). Expression of these structural genes requires a common set of regulatory genes and a common *cis*-acting DNA element. The positive regulatory genes include *INO2* and *INO4*, which encode basic helix–loop–helix (bHLH) proteins (14,15). The *INO2* and *INO4* gene products form a heterodimer that interacts with the UAS_{INO} element and is essential for *INO1* expression (14,16,17,18). Strains bearing mutant alleles of the *INO2* or *INO4* genes are inositol auxotrophs (19) because they are unable to derepress *INO1* transcription (10). In contrast, the products of the *OPI1* and *SIN3* regulatory genes act to repress the activities of the Ino2 and Ino4 proteins (20–22). Strains bearing mutant alleles of these negative regulators display an inositol excretion phenotype (Opi^+ phenotype) which correlates with overexpression of the *INO1* gene (23,24). In addition to the Opi^+ phenotype, these mutant strains constitutively overexpress the structural genes in the phospholipid biosynthetic pathway, and further experimental evidence indicates that the Opi1 and Sin3 repressors function through the only common *cis*-acting element found in these promoters, the UAS_{INO} element (22,25).

The *INO1* promoter includes a URS1 sequence which represses UAS_{INO}-driven expression of a *CYC1–lacZ* reporter gene (26). These observations prompted the present investigation of the role of the *UME6* gene in regulating transcription of the *INO1* gene. We report that the *UME6* gene was required for URS1-mediated repression of the *INO1* gene. Unexpectedly, the *UME6* gene was also required for induction of *CHO1*, *CHO2* and *OPI3* gene expression. The inability to induce expression of these genes in a *ume6*Δ mutant strain coincided with decreased activity of the *INO2* promoter. Therefore, these results provide the first evidence for a

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regulatory role for the *UME6* gene in phospholipid biosynthesis and in controlling expression of the regulatory gene *INO2*.

MATERIALS AND METHODS

Strains, media and culture conditions

The yeast strains used in this work are listed in Table 1. Strain BRS2005 was constructed by transformation of BRS1001 with a restriction fragment containing an *opi1Δ::LEU2* allele (25). Similarly, BRS2009 was constructed by transformation of BRS1001 with a restriction fragment containing a *ume6Δ::LEU2* allele (27). The diploid strain BRS1005 was homozygous for an *ino1-13* allele which conferred inositol auxotrophy. All yeast cultures used in this study were grown at 30°C in complete synthetic media containing 2% glucose (vol/vol) and either containing 75 μM inositol and 1 mM choline (I^+C^+) or lacking inositol and choline (I^-C^-) (10).

Opi$^+$ plate assay

Excretion of inositol was determined using a plate assay in a manner described previously (28). Briefly, yeast strains to be tested were patched onto plates lacking inositol and choline that had a reduced agar concentration (1.2%). The strains to be tested were allowed to grow for 72 h, and a suspension of the inositol auxotroph reporter strain BRS1005 was streaked away from the patch. Growth of the reporter strain was scored after an additional 72 h incubation at 30°C.

Enzyme assays

Yeast transformants were assayed for CAT activity as previously described (20). Units of CAT activity were defined as counts per minute measured in the organic phase and expressed as a percentage of total counts per minute (percent conversion) divided by the amount of protein assayed (in micrograms) and the time of incubation (in hours). Total protein concentration was determined using a Bio-Rad Protein Assay Kit (Bio-Rad, Rockville Center, NY).

RNA analyses

RNA was isolated from yeast using a glass-bead disruption and hot phenol extraction procedure (29). Northern and slot blot hybridizations were performed as described previously (10). Results were visualized by autoradiography and gene-specific c.p.m. quantitated using the Betascope 603 Blot Analyzer (Beta-gen, Waltham, MA). RNA probes (cRNA) for the Northern and slot blot hybridizations were synthesized using the Gemini II Core System (Promega, Madison, WI) from plasmids linearized with a restriction enzyme and transcribed with an RNA polymerase as follows (plasmid/restriction enzyme/RNA polymerase): pAB309Δ/*Eco*RI/SP6 (*TCM1*), pMH203/*Eco*RI/SP6 (*OPI3*), pAS103/*Hin*dIII/T7 (*CHO1*), pTG109/*Bam*HI/T7 (*CHO2*) and pJH310/*Hin*dIII/T7 (*INO1*).

Table 1. Yeast strains

Strain	Genotype	Source
BRS1001	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1*	This lab
BRS2005	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1, opi1::LEU2*	This study
BRS2009	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1, ume6::LEU2*	This study
BRS1005	MATa, *ade1, ino1-13*	This lab
	MAT α, *ade1, ino1-13*	
SFY59	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1, ade6*	C. Steber and R.E. Esposito
REE2276	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1, ade6,*	C. Steber and
	ime1::URA3	R.E. Esposito
BPA101	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This lab
	gal4::pBM-INO2::URA3	
BPA102	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This lab
	gal4::pBM-INO4::URA3	
JCJ101	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This lab
	gal4::pBM-INO1::URA3	
JCJ102	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This study
	gal4::pBM-INO1::URA3,ume6::LEU2	
JCJ103	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This study
	gal4::pBM-MURS::URA3	
JCJ104	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This study
	gal4::pBM-MURS::URA3, ume6::LEU2	
JCJ105	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This study
	gal4::pBM-INO2::URA3, ume6::LEU2	
JCJ106	MATa, *ade2-1, his3-11,15, leu2-3,112, can1-100, trp1-1, ura3-1,*	This study
	gal4::pBM-INO4::URA3, ume6::LEU2	

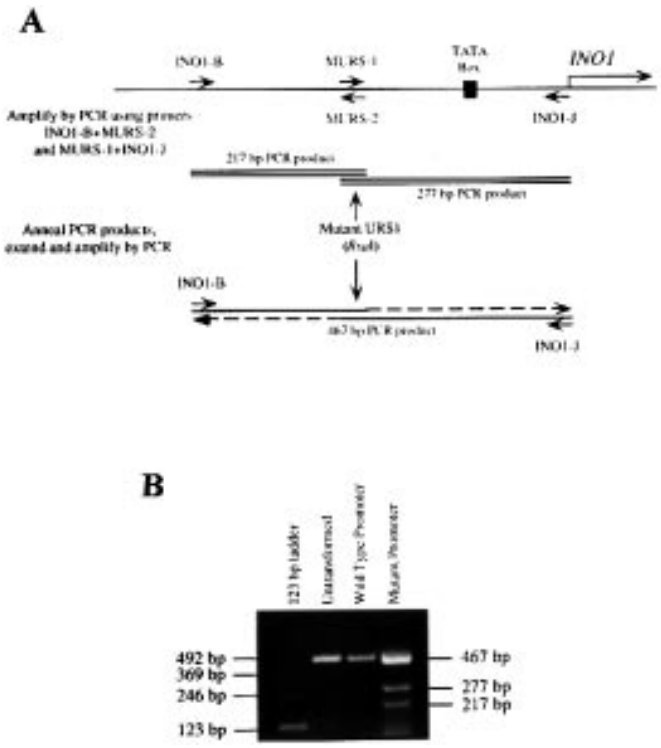

Figure 1. Construction of an *INO1* promoter fragment containing a mutant URS1 element. (**A**) Schematic depicting PCR mutagenesis of the *INO1* promoter. Two PCR fragments were generated which overlapped in the URS1 region. These PCR products were annealed, extended, and the full length *INO1* promoter fragment containing the mutated URS1 element was amplified by PCR using flanking primers INO1-B and INO1-J. The full-length PCR fragment was inserted upstream of the *cat* reporter gene as described previously (20). (**B**) Confirmation of the URS1 mutation in the pBM-MURS-*cat* reporter construct after integration into the yeast genome. Genomic DNA was isolated from an untransformed strain (BRS1001), a transformant containing an integrated wild-type *INO1* promoter–*cat* fusion, and a transformant containing an integrated pBM-MURS–*cat* fusion. The genomic DNA was used to amplify the region of the *INO1* promoter using the INO1-B and INO1-J primers. The resulting PCR products were purified and digested with *Rsa*I to confirm the presence of the mutated URS1 element. For reference is shown a 123 bp DNA ladder. The 467 bp band in the mutant promoter lane results from the native *INO1* promoter which contains a wild-type URS1 element and therefore lacks the *Rsa*I site.

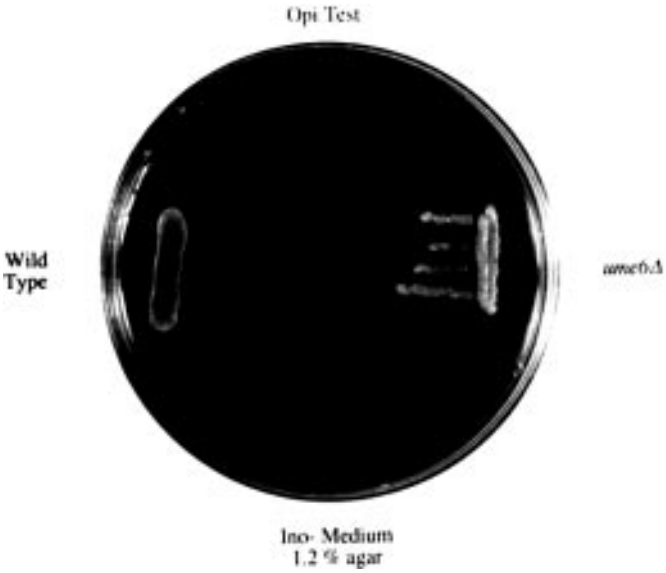

Figure 2. The *ume6*Δ mutant has an Opi$^+$ phenotype. Wild-type (BRS1001) and *ume6*Δ (BRS2009) strains were grown on media lacking inositol for 72 h at 30°C. The inositol auxotroph tester strain (BRS1005) was then streaked away from the patches and inositol cross-feeding scored after incubation at 30°C for 72 h.

Yeast transformations

Yeast strains were transformed using lithium acetate by a method previously described (30).

Plasmids

Plasmids pBM-INO2, pBM-INO4 and pBM-INO1 (used in the CAT assays) contained PCR-generated promoter sequences fused upstream of the *cat* reporter gene, and have been described in detail elsewhere (20). Plasmid pBM-MURS contained the portion of the *INO1* promoter found in pBM-INO1 (–453 to +1) with a PCR-generated mutant URS1 element replacing the native URS1 element. The mutant URS1 element was constructed using a previously described strategy (31) (Fig. 1A). Complimentary oligonucleotides, MURS1 (5′-CT**TCGTACGCT**AAATGCG-GC-3′) and MURS2 (5′-TT**AGCGTACGA**AGCGCATAC-3′), containing the desired mutation (*Rsa*I site) (underlined) in the URS1 element (bold) were synthesized. These were used in separate PCR reactions to generate PCR products that overlapped at the mutated URS1 element. These PCR products were purified, annealed and extended by *Taq* polymerase, and the resulting full-length promoter element was amplified using flanking oligos. Creation of the mutation in the URS1 element was verified by digestion with *Rsa*I. The mutated URS1 PCR product was cloned into the pGEM-T vector (Promega) to create pGEM-MURS. A *Bam*HI–*Bgl*II restriction fragment containing the *INO1* promoter with the mutant URS1 element was cloned into the *Bam*HI site of pBM2015 (20) creating pBM-MURS. Plasmid pBM-MURS was digested with *Cla*I and *Sst*II which liberated a fragment containing *GAL4* sequences flanking the promoter–*cat* fusion and the *URA3* selectable marker. Strains BRS1001 (wild type) and BRS2009 (*ume6*Δ) were transformed with this restriction fragment and uracil prototrophs were selected. Southern blot analysis confirmed integration of the reporter fusions at the *GAL4* locus in single copy. The presence of the *Rsa*I site (*i.e.* mutant URS1 element) was confirmed by isolating genomic DNA from the transformed strains, amplifying the *INO1* promoter region by PCR, and digesting the resulting PCR product with *Rsa*I. The amplified *INO1* promoter region was not digested by *Rsa*I in either the untransformed strains or the strains that contained the integrated wild-type *INO1* promoter–*cat* fusion, (Fig. 1B). In contrast, when DNA from the strains that contained the integrated mutant URS1 was used, three bands were observed after digestion with *Rsa*I (Fig. 1B). The larger band corresponded to the native *INO1* promoter, and the two smaller bands indicated the presence of the mutation in the URS1 element in the promoter–*cat* fusion at the *GAL4* locus.

RESULTS

A *ume6*Δ mutant strain had an Opi$^+$ phenotype

One class of regulatory mutants that affect phospholipid biosynthesis share the overproduction of inositol (Opi$^+$) phenotype which is

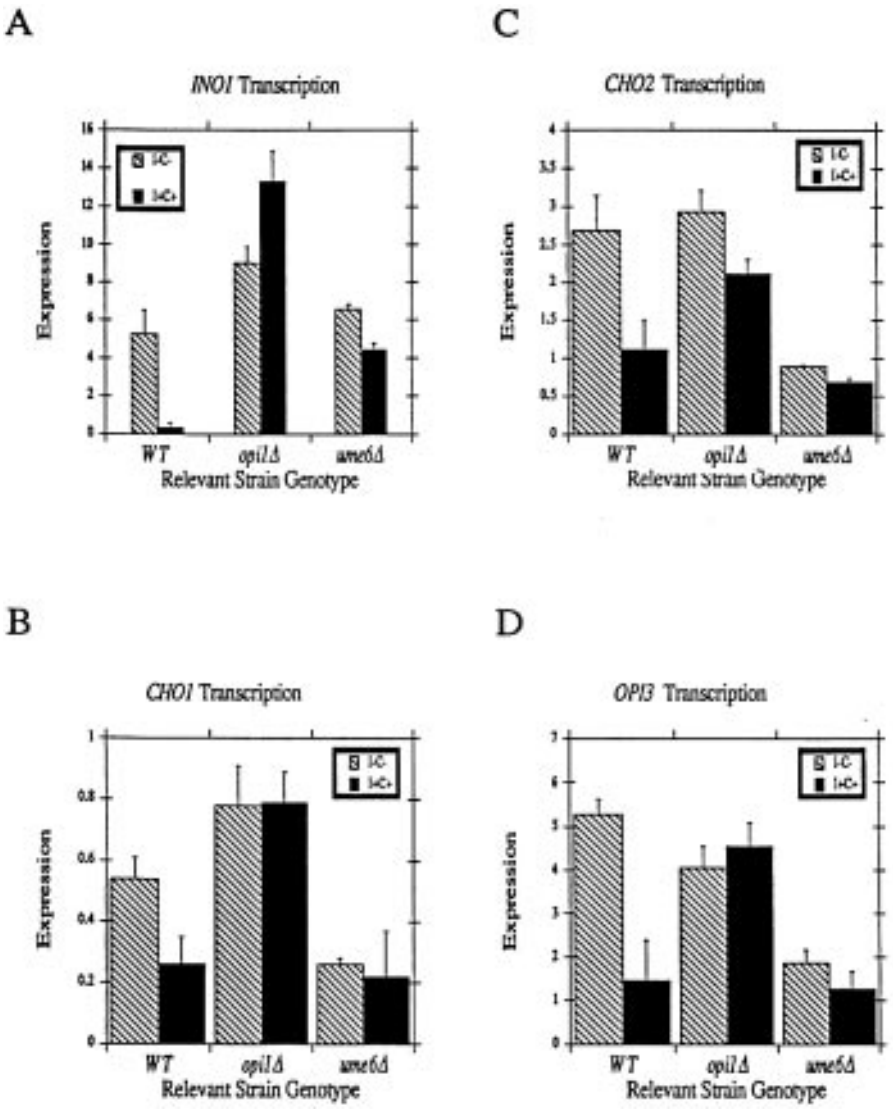

Figure 3. The *ume6*Δ mutation affects regulation of the phospholipid biosynthetic genes. The amount of *INO1* (**A**), *CHO1* (**B**), *CHO2* (**C**), and *OPI3* (**D**) transcript was determined by counting gene-specific c.p.m. of quantitative slot blots and normalized for loading variations using the constitutively expressed *TCM1* transcript (33). Each value represents the relative level of *INO1* expression from wild-type (BRS1001), *opi1*Δ (BRS2005) or *ume6*Δ (BRS2009) strains grown in media lacking (hatched) or containing (black) 75 μM inositol and 1 mM choline. Values represent the average of at least three independent assays, and standard deviations are indicated.

excretion of inositol into the growth media (23,24). In the case of the *opi1*Δ and *sin3*Δ mutants, this Opi$^+$ phenotype correlates with the constitutive overexpression of the *INO1* gene (21,25). Based on the presence of the URS1 element in the *INO1* promoter (26), it seemed plausible that *UME6* may be involved in repression of *INO1*. Therefore, a *ume6*Δ mutant strain may also display the Opi$^+$ phenotype. To examine this possibility, a wild-type (BRS1001) and a *ume6*Δ mutant strain (BRS2009) were patched onto media lacking inositol, and allowed to grow at 30°C for 3 days. After 3 days, a suspension of a diploid tester strain which is an inositol auxotroph (BRS1005) was streaked away from the original patches. The tester strain was expected to grow if inositol had been excreted into the media. This experiment showed that the *ume6*Δ mutant strain (BRS2009) did excrete inositol into the growth media, allowing for growth of the tester strain (BRS1005) (Fig. 2). As expected, the isogenic wild-type strain (BRS1001) did not support growth of the tester strain (BRS1005) (Fig. 2).

Regulation of phospholipid biosynthetic gene expression was altered in a *ume6*Δ mutant strain

The *SIN3* and *UME6* genes have been linked to URS1-mediated repression (21,26,7). Since a *sin3*Δ mutation has a pleiotropic effect on phospholipid biosynthetic gene expression and the products of the *UME6* and *SIN3* genes often function collectively, we examined if the *UME6* gene also had a role in controlling expression of the phospholipid biosynthetic genes. For this, total RNA was isolated from wild-type strain (BRS1001) and an isogenic *ume6*Δ mutant strain (BRS2009) grown in media lacking (derepressing) or containing inositol and choline (repressing). For

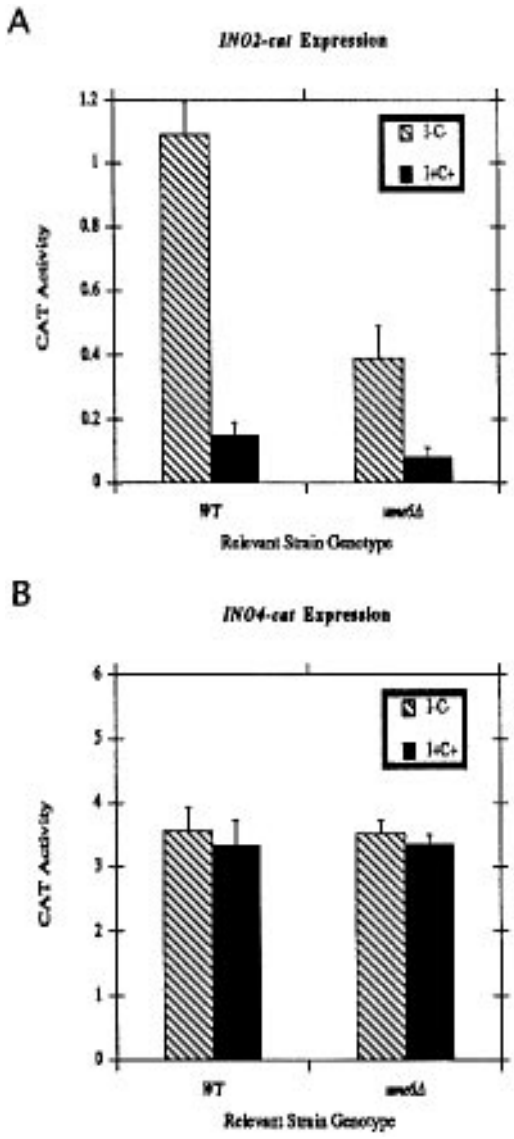

Figure 4. The *ume6*Δ mutation affects expression of the *INO2–cat* gene (**A**), but not the *INO4–cat* gene (**B**). CAT activity in wild-type (BRS1001) and *ume6*Δ (BRS2009) strains grown in media lacking (hatched) or containing (black) 75 μM inositol and 1 mM choline. Values represent the average of at least three independent assays, and standard deviations are indicated.

comparison, we also isolated RNA from an *opi1*Δ mutant strain (BRS2005). Expression of the phospholipid biosynthetic genes was quantitated by slot blot hybridization with appropriate cRNA probes, and normalized for loading variations to expression of the constitutive *TCM1* gene (32).

Since a strain harbouring a *ume6*Δ allele had the Opi$^+$ phenotype (Fig. 2), we first examined expression of the *INO1* gene because its overexpression typically correlates with the Opi$^+$ phenotype (25). Quantitation of *INO1* mRNA levels (Fig. 3) in these strain backgrounds demonstrated the different effects the negative regulators *OPI1* and *UME6* had on *INO1* gene expression. As has been shown previously, in the *opi1*Δ mutant background *INO1* was overexpressed in the presence or absence of inositol and choline (10,25) (Fig. 3). Contrastingly, in the *ume6*Δ background, the *INO1* gene was modestly overexpressed in derepressing conditions but its expression was still subject to regulation in the presence of inositol and choline (Fig. 3). Although, the degree of inositol-mediated repression was only ~30% in the *ume6*Δ strain (BRS2009).

As has been reported for the *sin3* mutant strain (21), we observed that expression of other phospholipid biosynthetic genes (*CHO1*, *CHO2* and *OPI3*) was also altered by the *ume6*Δ mutation (Fig. 3). In marked contrast to its effect on *INO1* gene expression, the *ume6*Δ mutation led to a significant decrease in the expression of the other phospholipid genes to wild-type repressed levels (Fig. 3). The *opi1*Δ mutation led to constitutive expression of these same genes at levels greater than or equal to those seen in the wild-type background under derepressing conditions (Fig. 3) which was similar to its effect on *INO1* expression (Fig. 3).

INO2 expression was altered in a *ume6*Δ mutant strain

The *ume6*Δ mutation eliminated derepression of *CHO1*, *CHO2* and *OPI3* gene expression (Fig. 3). This raised the possibility that the *ume6*Δ mutation had altered transcription of *INO2* and *INO4* activator genes. Previous work demonstrates that expression of the *INO2* transcriptional activator is regulated in the presence of inositol and choline in a manner similar to that of the other phospholipid biosynthetic genes (20). Based on these findings, we examined expression of *INO2* in the wild-type (BRS1001) and *ume6*Δ mutant (BRS2009) strains under repressing and derepressing conditions. For this, we used a plasmid that contains 500 basepairs of the sequence upstream of the AUG translation start codon of the *INO2* gene fused to a *GAL4–cat* fusion reporter (20). A single copy of this fusion was integrated into the yeast genome by homologous recombination at the *GAL4* locus. We found that expression of the *INO2–cat* reporter was dramatically reduced in the *ume6*Δ strain (BRS2009) as compared to the isogenic wild-type strain (BRS1001) (Fig. 4A). In the *ume6*Δ strain, CAT activity was reduced ~2-fold under repressing conditions, and reduced ~3-fold under derepressing conditions (Fig. 4A). Northern blot analysis confirmed that the *UME6* gene does regulate transcription of the *INO2* gene (data not shown).

Using the same strategy, we also tested whether expression of the *INO4* positive regulatory gene was altered in the *ume6*Δ strain. Previous work demonstrates that *INO4–cat* is constitutively expressed under both repressing and derepressing conditions (20). We observed that *INO4–cat* expression was unaffected by the *ume6*Δ mutation (Fig. 4B). Thus, *UME6* is required for proper regulation of *INO2* gene expression. This correlates with the observation that, of the two transcriptional activator genes, only *INO2* expression is regulated in response to inositol and choline (20).

Induction of *CHO1* gene expression is not dependent on the *IME1* gene

The Ume6-dependent induction of early meiotic genes requires the *IME1* gene (8), suggesting that the *IME1* gene might also be required for induction of *CHO1* gene expression. *CHO1* transcription was quantitated in a wild-type strain (SFY59) and an isogenic strain carrying an *ime1*Δ allele (REE2276). The data showed that *CHO1* transcription was unaffected by the *ime1*Δ mutant allele (data not shown). Similarly, transcription of the *INO2* gene was also not affected by the *ime1*Δ mutant allele (data not shown).

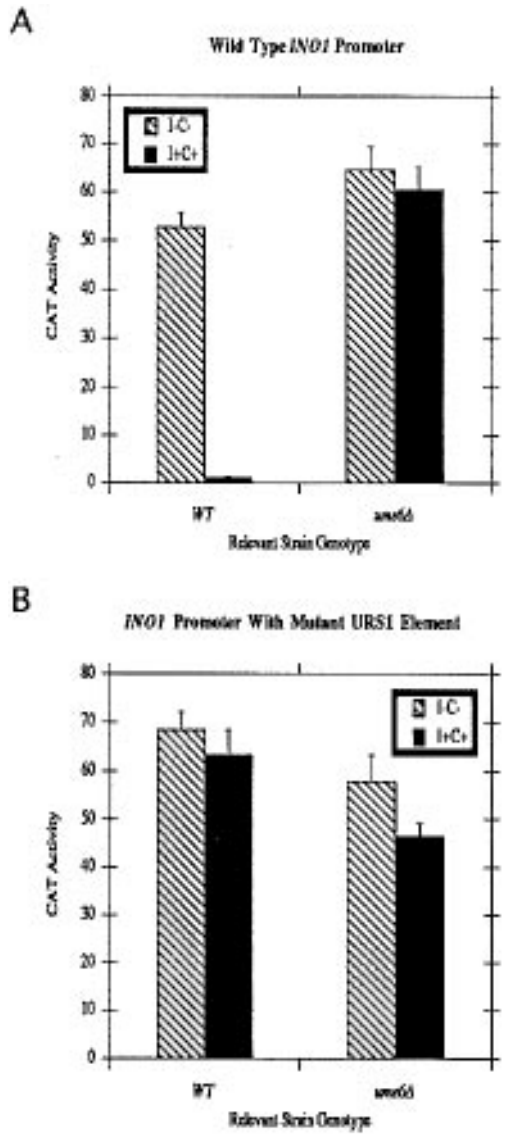

Figure 5. *UME6* represses *INO1* through the URS1 element. The effect of a *ume6*Δ mutant on expression from a wild-type *INO1* promoter (**A**) and an *INO1* promoter containing a mutant URS1 element (**B**). CAT activity in wild-type (BRS1001) and *ume6*Δ (BRS2009) strains grown in media lacking (hatched) or containing (black) 75 µM inositol and 1 mM choline. Values represent the average of at least three independent assays, and standard deviations are indicated.

UME6 exerted repression through the URS1 element found in the *INO1* promoter

Work on the *CAR1* gene demonstrated that the *UME6* gene was required for repression mediated by the URS1 element found in the *CAR1* promoter (33). The presence of a functional URS1 element in the *INO1* promoter (26) prompted us to examine the effect of a *ume6*Δ mutation on expression directed by an *INO1* promoter with a mutant URS1 element. For this analysis, we used wild type (BRS1001) and *ume6*Δ mutant (BRS2009) strains harbouring either a wild-type or URS1-mutant *INO1* promoter fused to the *cat* reporter gene. These strains were grown under repressing and derepressing conditions, and CAT activity was assayed.

When the *cat* construct containing the native *INO1* promoter was assayed (Fig. 5A), the pattern of regulation in the wild-type (BRS1001) and *ume6*Δ (BRS2009) strains was similar to the regulation of *INO1* transcript levels in these two strains (Fig. 3). That is, the *ume6*Δ mutation caused an increase in expression of the *INO1* gene (Fig. 3) and an increase in CAT activity that was not sensitive to the presence of inositol and choline (Fig. 5A). Mutating the URS1 element in the *INO1* promoter–*cat* fusion also led to constitutive CAT activity in both the wild-type (BRS1001) and *ume6*Δ (BRS2009) strains (Fig. 5B). The lack of synergy between the mutant URS1 and *ume6*Δ mutation, indicated that *UME6* exerts its repression on *INO1* expression through the URS1 element in the *INO1* promoter.

DISCUSSION

Repression of phospholipid biosynthesis in response to exogenous inositol and choline is a complex process involving at least two *cis*-acting sequences, the UAS_{INO} and the URS1 element and two *trans*-acting factors encoded by the *OPI1* and *SIN3* genes (21,26,34,25). Here, we report that the *UME6* negative regulatory gene was also required for proper regulation of the genes involved in phospholipid biosynthesis. Surprisingly, a *ume6*Δ mutation had disparate effects on expression of the genes involved in phospholipid biosynthesis. Among the structural genes in the phospholipid biosynthetic pathway, *INO1* expression was most dramatically affected. As is the case with mutations in the *SIN3* (21) or *OPI1* (25) genes, a *ume6*Δ mutant strain overproduced inositol and excreted it into the growth medium, indicating overexpression of the *INO1* gene (Fig. 2). Consistent with the Opi^+ phenotype, the *INO1* gene was overexpressed in a *ume6*Δ mutant strain, grown under repressing conditions (presence of inositol and choline), to levels seen in the wild-type strain under derepressing conditions (Fig. 3).

Mutations in either of the negative regulators, *OPI1* or *SIN3*, lead to constitutive expression of the other co-regulated structural genes in the phospholipid biosynthetic pathway, including the *CHO1*, *CHO2* and *OPI3* genes (Fig. 3) (21). By contrast, a *ume6*Δ mutation renders these genes constitutive, but at levels identical to those observed for a wild-type strain under repressed conditions (Fig. 3). Thus, our results identified a novel positive regulatory role for the *UME6* gene in controllling expression of the *CHO1*, *CHO2* and *OPI3* genes.

The positive regulatory role for the *UME6* gene on *CHO1*, *CHO2* and *OPI3* expression suggested that *UME6* may have been required for proper expression of the *INO2* and *INO4* transcriptional activator genes. It is known that expression of an *INO2–cat* fusion gene is regulated in response to inositol and choline, while expression of an *INO4–cat* fusion construct is known to be constitutive (20). Moreover, in an *opi1*Δ mutant, the *INO2–cat* fusion gene is constitutively overexpressed whereas expression of the *INO4–cat* gene is unaffected (20). In contrast to the *opi1*Δ effect, *INO2–cat* expression in a *ume6*Δ mutant strain was markedly decreased under both repressing and derepressing conditions when compared to a wild-type strain (Fig. 4). Thus, the *UME6* gene had a positive regulatory role in transcription from the *INO2* promoter. This decreased expression of the *INO2* activator gene in the *ume6*Δ strain can explain the effect of the *ume6*Δ mutation on expression of the *CHO1*, *CHO2* and *OPI3* genes. We

have previously shown that *INO2* gene expression is required for increased expression of the *CHO1* gene when cells are grown under derepressing conditions (35). Thus, at low levels of *INO2* gene expression, *CHO1* gene expression becomes nearly unresponsive to inositol and choline supplementation. Therefore, we suggest that the severe reduction in *INO2* expression in the *ume6*Δ mutant strain was responsible for eliminating repression of *CHO1, CHO2* and *OPI3* gene expression by inositol and choline.

One parsimonious explanation for the reduction in *INO2–cat* expression lies in the ability of a *ume6*Δ mutant strain to overproduce inositol and excrete it into the growth media (Fig. 2). The *ume6*Δ mutation may cause a significant rise in the internal pools of inositol, resulting in repression of *INO2–cat* expression; however, we do not favour this explanation based on the following observation. A strain harbouring a mutation in the general transcriptional repressor *sin3* also overexpresses the *INO1* gene and excretes inositol while expressing the *CHO1, CHO2* and *OPI3* genes at derepressed levels (21), indicating that *INO2* expression is not repressed by excess inositol.

Many yeast genes in unrelated systems are known to contain a URS1 element in their promoters, and to require this element for repression of gene expression. In this report, we directly examined the role of the URS1 element in repression of *INO1* gene expression. We created two fusions of the *INO1* promoter to the *cat* reporter gene, which were identical except for a mutation of the URS1 element of one reporter construct. The reporter constructs containing the mutation in the URS1 element gave constitutive CAT activity (Fig. 5) regardless of strain genotype, indicating that the URS1 element is crucial for repression of *INO1*. Curiously, the wild-type strain gave levels of CAT activity that were higher than in the *ume6*Δ strain. This effect may be due to the lower expression of the *INO2* activator gene in the *ume6*Δ strain (Fig. 4). Since there was no synergy between the *ume6*Δ mutation and the mutant URS1 element, we concluded that *UME6* regulates *INO1* gene expression primarily through the URS1 element. Regulation involving the URS1 element is quite complex and can involve several different system-specific players. In the case of the *CAR1* gene, which is involved in nitrogen metabolism, the *UME6* gene is absolutely required for URS1-mediated repression (7), although it is the products of the *BUF1* and *BUF2* (*RPA1* and *RPA2*) genes that actually bind to the URS1^{CAR1} element (33). However, in the case of the meiosis-specific gene *SPO13*, experiments using an MBP–Ume6 fusion protein have demonstrated direct binding of the Ume6 fusion protein to the URS1^{SPO13} element (27). In addition, full repression of the *SPO13* gene also requires the product of the *SIN3* gene (36), which is not required for repression of *CAR1* (7).

Six different systems of repression using the *SIN3* and *UME6* regulatory genes and the URS1 element can now be defined. There are systems (e.g. *SPO13* and *INO1*) that use both *SIN3* and *UME6* as repressors through a URS1-dependent pathway (22,27; and results presented here). There are systems that use either *SIN3* (e.g. *HO*) (37) or *UME6* (e.g. *CAR1*) (7) as repressors through URS1-dependent pathways. There are also systems that use *SIN3*, as a repressor, but are URS1-independent (e.g. *TRK2* and *INO1*) (22,38). In addition, the *UME6* gene product has been shown to be required as a URS1-dependent activator of meiotic genes during meiotic development (8,9). Finally, our results demonstrated that the *UME6* gene controls phospholipid biosynthetic gene expression through a mechanism that involved stimulation of expression of the *INO2* activator gene but is URS1-independent.

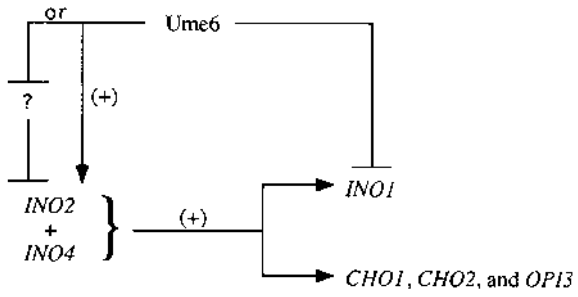

Figure 6. Model for the role of the *UME6* gene product in the regulation of phospholipid biosynthetic genes. Refer to Discussion for a complete description of the model.

We propose a model to explain the role of the *UME6* gene product on expression of the phospholipid biosynthetic genes (Fig. 6). The model predicts that the Ume6 protein functions to directly inhibit transcription of the *INO1* gene and that it may stimulate transcription of the *INO2* gene either directly or indirectly. The direct mechanism would require that Ume6 function as a transcriptional activator of the *INO2* gene. This mechanism is difficult to envision since the *INO2* promoter lacks any URS1-like sequences and since Ume6 was not capable of activating transcription in a diploid cell during vegetative growth (8). Therefore, this mechanism would require that Ume6 function as a URS1-independent, *IME1*-independent, haploid-specific transcriptional activator. We currently favour the indirect mechanism which predicts that Ume6 would function to repress a negative regulator of *INO2* transcription. This indirect mechanism would not require the presence of a URS1 element in the *INO2* promoter and would not be dependent on the *IME1* gene.

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