

# Regulation of yeast phospholipid biosynthetic gene expression in response to inositol involves two superimposed mechanisms

(activators/basic-helix-loop-helix proteins/repressor/weak promoters/*Saccharomyces cerevisiae*)

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**ABSTRACT** Transcription of phospholipid biosynthetic genes in the yeast *Saccharomyces cerevisiae* is maximally derepressed when cells are grown in the absence of inositol and repressed when the cells are grown in its presence. We have previously suggested that this response to inositol may be dictated by regulating transcription of the cognate activator gene, *INO2*. However, it was also known that cells which harbor a mutant *opi1* allele express constitutively derepressed levels of target genes (*INO1* and *CHO1*), implicating the *OPI1* negative regulatory gene in the response to inositol. These observations suggested that the response to inositol may involve both regulation of *INO2* transcription as well as *OPI1*-mediated repression. We investigated these possibilities by examining the effect of inositol on target gene expression in a strain containing the *INO2* gene under control of the *GAL1* promoter. In this strain, transcription of the *INO2* gene was regulated in response to galactose but was insensitive to inositol. The expression of the *INO1* and *CHO1* target genes was still responsive to inositol even though expression of the *INO2* gene was unresponsive. However, the level of expression of the *INO1* and *CHO1* target genes correlated with the level of *INO2* transcription. Furthermore, the effect of inositol on target gene expression was eliminated by deleting the *OPI1* gene in the *GAL1-INO2*-containing strain. These data suggest that the *OPI1* gene product is the primary target (sensor) of the inositol response and that derepression of *INO2* transcription determines the degree of expression of the target genes.

Regulation of gene expression in yeast has been extensively documented (reviewed in refs. 1 and 2) and several well-defined systems have emerged as models for how the yeast cell responds to environmental signals by coordinately varying gene transcription (3–5). These model systems have identified specific interactions between cis-acting upstream activation sequences (UASs) (6) and their cognate trans-acting regulatory proteins. Recent investigations have focused on understanding the role(s) of trans-acting regulatory proteins in coordinating gene expression. These roles generally fall into two broad categories. The first category includes regulation of the amount of functional activator—e.g., regulation of *GAL4* transcription in response to glucose (7), of *GCN4* translation (8) and *Gcn4* protein stability (9) in response to amino acid starvation, and of *Swi5* and *Ace2* transit into the nucleus (10, 11). The second category invokes repressors that specifically interact with activators to inhibit their function—e.g., modulation of the interaction between the Gal80 repressor and the Gal4 activator (12) or between the Pho80 repressor and the Pho4 activator (13). However, it is unusual to find a system that invokes both categories in response to a single environmental cue. This report examines the regulation of phospholipid biosynthetic gene expression in response to inositol. We show

that the response to inositol involves both transcriptional regulation of the *INO2* activator gene and the action of the *OPI1* negative regulatory gene.

Transcription of the phospholipid biosynthetic genes is maximally derepressed in the absence of inositol and repressed in its presence (reviewed in refs. 14 and 15). A highly conserved 10-bp element (5'-CATGTGAAAT-3') found in the promoters of the coregulated genes has been shown to be both necessary and sufficient for the inositol response (16, 17). This element (*UAS<sub>INO</sub>*) includes the canonical binding site for the basic-helix-loop-helix (bHLH) family of proteins (5'-CANNTG-3') (14, 18, 19). Thus, it was not surprising to find that the *UAS<sub>INO</sub>* sequence serves as a binding site for a heterodimer composed of two bHLH proteins, Ino2 and Ino4 (20–22). Consistent with their predicted role as transcriptional activators, the *INO2* and *INO4* genes have been shown to be indispensable for derepression of phospholipid biosynthetic gene expression in response to inositol deprivation (14, 15).

Recently, we showed that expression of an *INO2* promoter-chloramphenicol acetyltransferase (CAT) fusion gene was regulated in response to inositol in a pattern that was indistinguishable from that of its target genes (23). This observation suggested that regulation of the phospholipid biosynthetic genes in response to inositol may involve regulation of transcription of the *INO2* activator gene. However, regulation of phospholipid biosynthetic gene expression is also dependent on a negative-acting regulatory gene, *OPI1* (24). Strains that harbor null alleles of *OPI1* constitutively overexpress the phospholipid biosynthetic structural genes (24) as well as the *INO2* gene (23). Therefore, the response to inositol may involve both categories of mechanisms—i.e., regulation of expression of the *INO2* activator gene and repression by the *OPI1* gene product (Fig. 1 *Upper*). However, we could not preclude the possibility that the role of *OPI1* might be to regulate *INO2* gene expression (Fig. 1 *Lower*). To distinguish between these two models we uncoupled *INO2* expression from the inositol response by placing it under the control of the *GAL1* promoter. In a strain that contains the *GAL1-INO2* fusion, expression of the target genes (*INO1* and *CHO1*) was found to be regulated in response to both inositol and galactose concentrations. However, the inositol response was eliminated when the *OPI1* gene was deleted in this same strain.

## MATERIALS AND METHODS

**Strains and Growth Conditions.** Yeast strains used in this study were BRS1001 (*MAT $\alpha$ , ade2-1, his3-11,15, leu2-3,112, can1-100, ura3-1, trp1-1*), BRS2002 (*MAT $\alpha$ , ade2-1, his3-11,15, leu2-3,112, can1-100, ura3-1, trp1-1, ino2 $\Delta$ ::TRP1*), and BRS2005 (*MAT $\alpha$ , ade2-1, his3-11,15, leu2-3,112, can1-100, ura3-1, trp1-1, opi1 $\Delta$ ::LEU2*), BRS2011 (*MAT $\alpha$ , ade2-1, his3-*

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Abbreviations: CAT, chloramphenicol acetyltransferase; UAS, upstream activation sequence.

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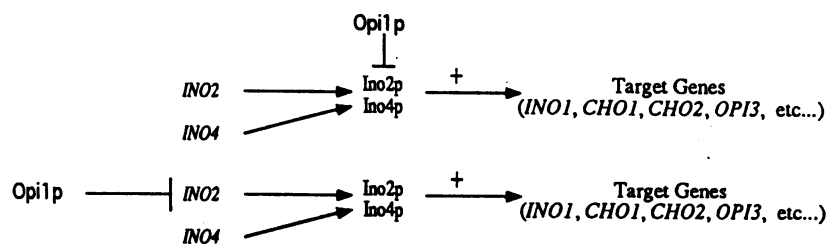


FIG. 1. Models depicting potential regulatory cascades for phospholipid biosynthetic gene expression. Genes are designated in italics and proteins by the "p" suffix. Refer to the text for a complete description.

11,15, *leu2-3,112*, *can1-100*, *ura3-1::pGAL1-INO2::URA3*, *trp1-1*, *ino2Δ::TRP1*), and BRS2012 (*MATα*, *ade2-1*, *his3-11,15*, *leu2-3,112*, *can1-100*, *ura3-1::pGAL1-INO2::URA3*, *trp1-1*, *ino2Δ::TRP1*, *opi1Δ::LEU2*). The construction of strains containing the *INO2-cat* reporter gene integrated in single copy at the *GAL4* locus has been previously described (23). All cultures were grown at 30°C in synthetic medium (25) either supplemented with 75 μM inositol and 1 mM choline or lacking inositol and choline. Media containing different carbon sources are described in the text.

**Plasmid Construction and Chromosomal Integration.** Plasmid pBM2289 (26) was used to fuse the *INO2* coding sequence to the *GAL1* promoter. This plasmid contains the wild-type *GAL1* promoter, upstream of an *Sph* I restriction site, and the *URA3* selectable marker. The *INO2* gene was amplified by PCR using a 5' primer (5'-GCATGCATGCAACAAGCAACT-3') which included the translational initiator codon for the *INO2* gene flanked by an *Sph* I restriction site for subcloning purposes. The 3' PCR primer (5'-GATCATTG-CACCGTT-3') was targeted to sequences downstream of the translational stop codon for the *INO2* gene. This was done to ensure that sequences important for RNA 3'-end maturation were included. The *INO2* PCR product was cloned into the pGEM-T vector (Promega) to create pGEM-*INO2*. An *Sph* I restriction fragment containing the *INO2* coding sequence was cloned into an *Sph* I restriction site in pBM2289, creating pGAL1-*INO2*. The pGAL1-*INO2* construct places the *INO2* coding DNA immediately downstream of the wild-type *GAL1* promoter. The pGAL1-*INO2* plasmid was linearized within the *URA3* gene (*Stu* I) and used to transform an *ino2* deletion mutant strain (BRS2002), to create BRS2011. Single-copy integrants at the *URA3* locus were confirmed by Southern blot analysis. A derivative of BRS2011 that contained a null allele of the *OPI1* gene (*opi1Δ::LEU2*) was constructed by transformation with a restriction fragment carrying the *opi1Δ* null allele (24) and was designated BRS2012.

**RNA Analyses.** RNA was isolated from yeast by a glass-bead disruption/hot phenol extraction procedure (27). RNA probes for Northern and quantitative slot blot hybridizations (23, 25) were synthesized with the Gemini II core system (Promega) from plasmids linearized with a restriction enzyme as follows (shown as plasmid, restriction enzyme, RNA polymerase) for the indicated (parenthesized) probe: pGEM-*INO2*, *Sal* I, T7 (*INO2*); pPLg, *Bam*HI, SP6 (*ACT1*). Probes for *INO1*, *CHO1*, and *TCM1* have been described (23). The results of Northern and slot blot hybridizations were visualized by autoradiography and quantitated by densitometry.

**CAT Enzyme Assays.** CAT activity was determined with a phase-extraction procedure (7, 23). Units of CAT activity were defined as counts per minute measured in the organic phase and expressed as a percentage of the total counts per minute (percent conversion) divided by the amount of protein assayed (in micrograms) and the time of incubation (in hours).

## RESULTS

**Uncoupling *INO2* Transcription from the Inositol Response.** Expression of the *cat* reporter gene driven by the *INO2*

promoter (integrated in single copy at the *GAL4* locus in BRS1001) (23) was sensitive to different inositol concentrations in the growth medium (Fig. 2). Specifically, we observed increased levels of CAT activity with decreasing concentrations of inositol. The effect of the different inositol concentrations on expression of the *INO2-cat* gene was similar to the effect on expression of the *INO2*-target genes *INO1* and *CHO1* (23, 25, 28). This suggested that regulation of *INO2* expression may be the primary mechanism for the coordinated response to inositol. To directly determine the role of *INO2* expression in the regulation and/or expression of the target genes, we uncoupled *INO2* expression from the inositol response by placing it under the control of the galactose-inducible *GAL1* promoter. To do this, we constructed a plasmid (pGAL1-*INO2*) that placed the *INO2* coding sequence downstream of the *GAL1* promoter in plasmid pBM2289 (26). Plasmid pGAL1-*INO2* (containing the *URA3* selectable marker) was stably integrated in single copy at the *ura3* locus of strain BRS2002 (*ino2Δ*) to yield BRS2011 (pGAL1-*INO2::URA3*, *ino2Δ*). We chose to use BRS2002 because it contained a deletion allele of the *INO2* gene and therefore ensured that *INO2* expression originated exclusively from the *GAL1-INO2* hybrid gene.

Expression of the *INO2* gene in BRS2011 (pGAL1-*INO2::URA3*, *ino2Δ*) was expected to be sensitive to carbon source (*GAL1* promoter-driven) but insensitive to inositol. We tested this prediction by using two assays for *INO2* expression. First, we compared the growth phenotype of BRS2011 (pGAL1-*INO2::URA3*, *ino2Δ*) on media containing different carbon sources and either lacking or containing inositol (Table

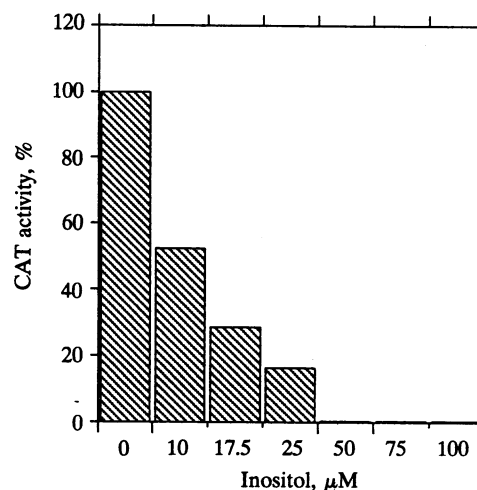


FIG. 2. Expression of the *INO2*-promoter driven CAT reporter gene is sensitive to inositol concentration. CAT activity was assayed from extracts of wild-type cells (BRS1001) containing a single copy of the reporter gene integrated in single copy at the *GAL4* locus (23). Cells were grown in media containing various concentrations of inositol. All values are presented as a percentage of completely derepressed levels and are the average of at least three independent assays. Standard deviations were less than 15% in all cases.

Table 1. Growth phenotype of *GAL1-INO2*-containing *ino2Δ* strain

Strain (genotype)	Inositol			No inositol		
	Gal	Raf	Glc	Gal	Raf	Glc
BRS1001 ( <i>INO2</i> )	+	+	+	+	+	+
BRS2002 ( <i>ino2Δ</i> )	+	+	+	-	-	-
BRS2011 (pGAL1- <i>INO2::URA3, ino2Δ</i> )	+	+	+	+	±	-

Strains were tested by spotting  $\approx 10^6$  cells on complete synthetic medium (21) that was either supplemented with 75  $\mu\text{M}$  inositol or lacked inositol and that contained either 2% galactose (Gal), 2% raffinose (Raf), or 2% glucose (Glc) by weight. Growth was scored after 48 hr (30°C) as wild type (+), no growth (-), or slow growth ( $\pm$ ).

1). BRS2011 (pGAL1-*INO2::URA3, ino2Δ*) grew normally on galactose-containing medium regardless of the presence or absence of inositol. That is, the level of *GAL1* promoter-driven *INO2* gene expression in medium containing galactose rescued the inositol auxotrophy associated with the *ino2Δ* mutant

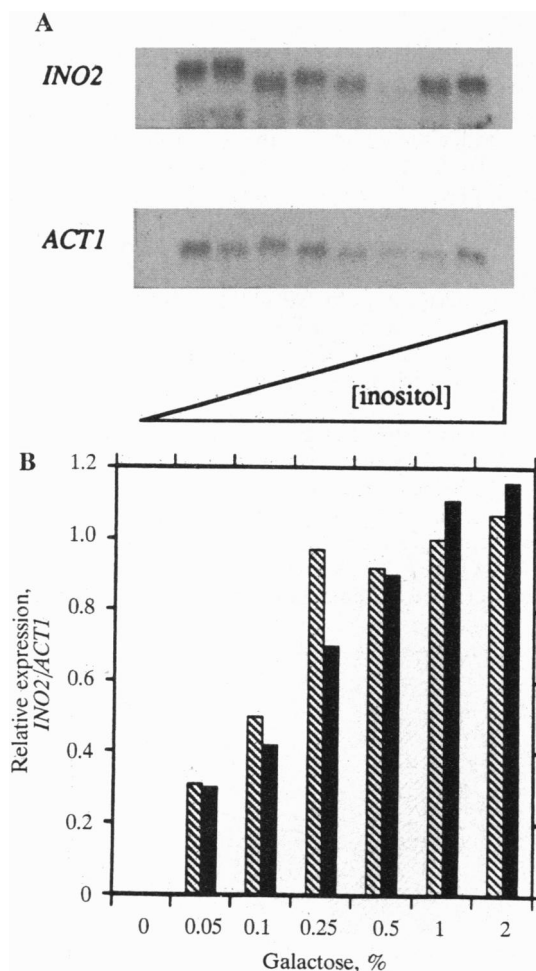


FIG. 3. Uncoupling *INO2* expression from the inositol response. (A) Expression of *INO2* transcript from strain BRS2011 (pGAL1-*INO2::URA3, ino2Δ*) grown in media containing 0.5% galactose and various concentrations of inositol (from left to right: 0, 5, 10, 17.5, 25, 50, 75, and 100  $\mu\text{M}$ ). The same blot was rehybridized with the *ACT1*-specific probe to normalize for loading variations. (B) Relative levels of *INO2* transcription (arbitrary densitometry units) from BRS2011 (pGAL1-*INO2::URA3, ino2Δ*) grown in media containing various concentrations of galactose either lacking (hatched bars) or containing (solid bars) 75  $\mu\text{M}$  inositol and 1 mM choline. The amount of *INO2* transcript was determined by densitometric scanning of quantitative slot blots and normalized for loading variations by using the *ACT1* transcript. Values represent the average of three independent assays. Standard deviations were less than 15% in all cases.

allele. However, this same strain grew slowly on a raffinose medium, and failed to grow on a glucose medium when inositol was omitted. The inability of BRS2011 (pGAL1-*INO2::URA3, ino2Δ*) to grow on glucose and grow slowly on raffinose is due to expression from the *GAL1* promoter, which is severely repressed when cells are grown on glucose-containing medium and reduced on raffinose-containing medium (3). Consequently, *INO2* expression may be limiting under these two growth conditions, which would affect the ability of the *ino2Δ* strain to grow in the absence of inositol. As controls, we also examined the growth of an isogenic *INO2* strain (BRS1001) and the isogenic parental strain carrying the *ino2Δ* allele (BRS2002). As expected, the *INO2* wild-type strain grew under all conditions whereas the *ino2Δ* strain required inositol for growth regardless of the carbon source (Table 1).

The second assay involved direct quantitation of *INO2* transcription in BRS2011 (pGAL1-*INO2::URA3, ino2Δ*) by Northern and slot blot hybridizations. For this, we grew cells in media that contained different concentrations of galactose and either lacked or contained inositol. *INO2* expression from the *GAL1* promoter was not sensitive to the presence of inositol in the growth medium (Fig. 3 A and B) but was sensitive to the concentration of galactose in the medium (Fig. 3B). The presence of different concentrations of galactose had previously been shown to result in different levels of expression from the *GAL1* promoter (29). Consequently, we uncoupled *INO2* expression from the inositol response and made it sensitive to galactose concentration.

**Transcription of the *INO2* Gene Correlates with Transcription of Its Target Genes.** The BRS2011 strain (pGAL1-*INO2::URA3, ino2Δ*) allowed us to determine whether regulation of *INO2* expression is a component of the coordinated response to inositol. That is, does yeast coordinately derepress expression of the phospholipid biosynthetic genes in response to inositol by simply derepressing expression of the *INO2* gene? To address this question we directly quantitated transcription of two *INO2*-target genes, *INO1* (25) and *CHO1* (28), in BRS2011 (pGAL1-*INO2::URA3, ino2Δ*) grown in media containing varying concentrations of galactose in both the presence and the absence of inositol. Transcription of the *INO1* gene in BRS2011 (pGAL1-*INO2::URA3, ino2Δ*) was sensitive to both galactose and inositol in the growth medium (Fig. 4). That is, in the absence of inositol, transcription of the *INO1* gene correlated with the concentration of galactose in the growth medium. However, in the presence of inositol, *INO1* transcription was repressed regardless of the galactose concentration. Similarly, transcription of the *CHO1* target gene was also

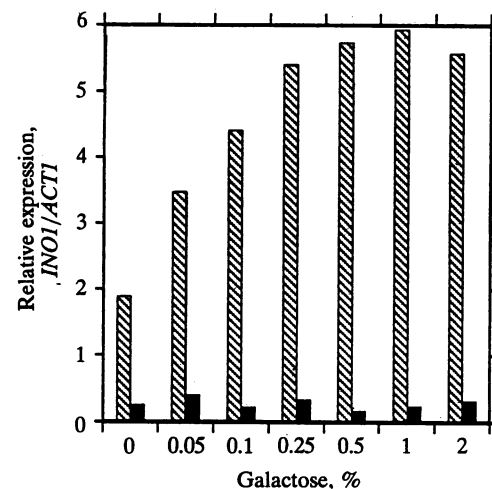


FIG. 4. Transcription of the *INO1* gene is sensitive to both galactose concentration and inositol in the *GAL1-INO2*-containing strain. Data were generated as described in the legend to Fig. 3B.

sensitive to both galactose and inositol (Fig. 5). Thus, in BRS2011 (pGAL1-*INO2*::*URA3*, *ino2Δ*), transcription of the *INO1* and *CHO1* target genes was still repressed in response to inositol supplementation even though *INO2* transcription was no longer sensitive to inositol (Fig. 3). Because the *GAL1* promoter is significantly stronger than the *INO2* promoter, we conducted a set of experiments parallel to those shown in Fig. 4, by expressing the *INO2* gene under control of the weak *GAL4* promoter. The *GAL4* promoter is about twice as strong as the *INO2* promoter (unpublished observations) and is repressed when cells are grown in glucose-containing medium (7). We obtained the same results with the *GAL4-INO2*-containing strain as we report here with the *GAL1-INO2* strain. That is, *INO1* gene expression was still subject to regulation by inositol even though *INO2* expression was now under control of a glucose-repressible promoter (7). However, it was not possible to confirm that *INO2* expression driven by the *GAL4* promoter was uncoupled from the inositol response, because of the weakness of the *GAL4* promoter (ref. 7; unpublished observations).

Thus, the coordinated response to inositol does not appear to be exclusively dictated by controlling *INO2* expression. However, the degree of derepression of the *INO1* and *CHO1* genes did correlate with the level of *INO2* transcription in BRS2011 (pGAL1-*INO2*::*URA3*, *ino2Δ*). That is, there was a correlation between the level of expression of the *INO2* activator gene (Fig. 3B) and the target genes at galactose concentrations between 0 and 0.5% (Figs. 4 and 5). However, while *INO2* transcription continued to increase at galactose concentrations greater than 0.5% (Fig. 3B), *INO1* and *CHO1* transcription did not increase under these same growth conditions (Figs. 4 and 5).

**The *OPI1* Gene Is Required for the Inositol Response in a *GAL1-INO2* Strain.** Since regulation of *INO2* gene transcription was not the primary target of the inositol response, we reasoned that the *OPI1* negative regulatory gene might be the primary target. This line of reasoning was supported by the phenotype of strains carrying *opi1* mutant alleles. In an *opi1* mutant strain, expression of the *INO1* (24, 25) and *CHO1* (28) target genes is insensitive to the presence of inositol in the growth medium. This suggests that the product of the *OPI1* gene either regulates *INO2* expression (Fig. 1 Upper) or directly regulates the function of the Ino2 protein (Fig. 1 Lower). To distinguish between these two models, we examined the effect of deleting the *OPI1* gene in BRS2011

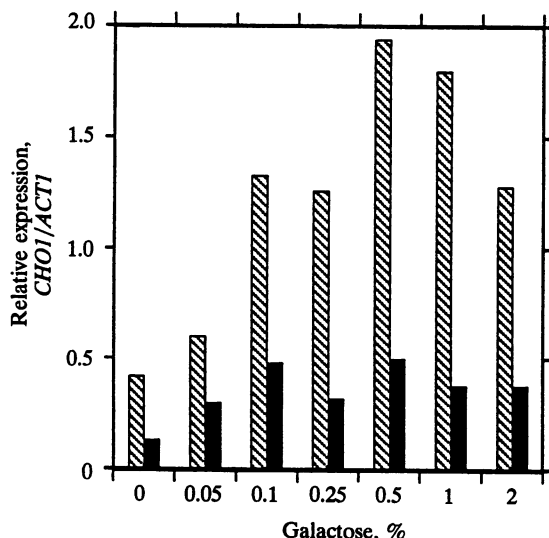


FIG. 5. Transcription of the *CHO1* gene is sensitive to both galactose concentration and inositol in the *GAL1-INO2*-containing strain. Data were generated as described in the legend to Fig. 3B.

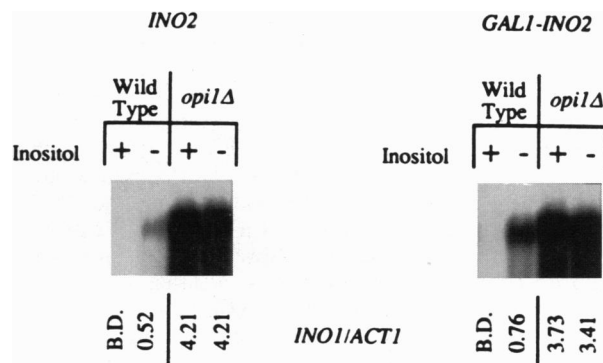


FIG. 6. Effect of an *opi1Δ* allele on *INO1* expression in the pGAL1-*INO2*-containing strain. Representative Northern blot analysis of *INO1* transcript in strains containing *INO2* and *OPI1* (BRS1001), *INO2* and *opi1Δ* (BRS2005), pGAL1-*INO2*::*URA3* and *OPI1* (BRS2011), or pGAL1-*INO2*::*URA3* and *opi1Δ* (BRS2012). Each strain was grown in medium containing 0.5% galactose which either lacked (-) or was supplemented with 75  $\mu$ M inositol and 1 mM choline (+). The values below each lane represent relative levels of *INO1* transcript (normalized for loading by using the *ACT1* transcript) determined by densitometry. Shorter exposures were used for densitometric scanning of RNA from the *opi1Δ* strains. B.D., below detection.

(pGAL1-*INO2*::*URA3*, *ino2Δ*) on regulation of *INO1* gene expression. If the response to inositol was mediated by Opi1 regulating the function of Ino2 (Fig. 1 Lower), then deletion of *OPI1* gene in BRS2011 (pGAL1-*INO2*::*URA3*, *ino2Δ*) should yield constitutive expression of the *INO1* target gene.

To directly examine the role of the *OPI1* gene in the response to inositol we deleted the *OPI1* gene in strain BRS2011 (pGAL1-*INO2*::*URA3*, *ino2Δ*) to yield BRS2012 (pGAL1-*INO2*::*URA3*, *opi1Δ*, *ino2Δ*). We then examined the effect of the *opi1Δ* allele on regulation of *INO1* expression in BRS2012 (pGAL1-*INO2*::*URA3*, *opi1Δ*, *ino2Δ*) by Northern blot hybridization. Total RNA was purified from strains grown in media that contained 0.5% galactose and either lacked or included inositol. The level of *INO1* transcripts was quantitated by densitometry and normalized for loading by use of the *ACT1* gene probe. As expected, BRS2012 (pGAL1-*INO2*::*URA3*, *opi1Δ*, *ino2Δ*) expressed constitutively elevated levels of *INO1* relative to the isogenic BRS2011 strain (pGAL1-*INO2*::*URA3*, *ino2Δ*) (Fig. 6 Right). In fact, the pattern of regulation in the strains containing pGAL1-*INO2* (Fig. 6 Right) was virtually indistinguishable from that in the strains containing the native *INO2* gene (Fig. 6 Left).

## DISCUSSION

We have determined that the response to inositol requires two superimposed mechanisms. One mechanism is the regulation of *INO2* activator gene expression, which is subject to auto-regulation by the *INO2* gene product (23). The second mechanism requires the product of the *OPI1* negative regulator, which may function as a direct regulator of Ino2/Ino4 activity (Fig. 1 Upper). Our data favor a model wherein the *OPI1* gene product (Opi1) is the primary target of the inositol response. We had previously shown that Opi1 is required to regulate expression of the *INO2* activator gene (23). However, here we show that it is also required to directly regulate expression of the *INO1* target gene (Fig. 6). These observations are paradoxical because both mechanisms are in operation in a wild-type yeast. Thus, it is difficult to determine if the primary role of the *OPI1* gene product is to regulate target gene expression directly or indirectly through regulation of *INO2* regulatory gene expression. In part, the resolution of this paradox is

dependent on determining how much Ino2 protein is present in the cell under various growth conditions.

Nevertheless, it is clear that *OPII* is absolutely required for the inositol response, whereas regulation of *INO2* expression can be eliminated without affecting regulation of the target genes in response to inositol (Figs. 4 and 5). Furthermore, *OPII* seems a likely target for the inositol response, since it appears to be expressed at a level higher than either *INO2* or *INO4* (23). We have previously shown that the *OPII* promoter is capable of driving constitutive expression (i.e., unresponsive to inositol) of a *cat* reporter gene at a level that is substantially higher than either the *INO2* or *INO4* promoters (23). Curiously, the relative levels of expression of the *OPII* and *INO2* regulatory genes (23) are reminiscent of the relative levels of *GAL80* and *GAL4* expression (26, 30). Consistent with this line of reasoning, it has been proposed that the *GAL80* gene product is the sensor for the intracellular inducer of the *GAL* system (31).

We observed a strong correlation between *INO2* expression driven by the *GAL1* promoter and expression of two target genes, *INO1* and *CHO1*. This suggests that regulation of *INO2* expression does play a role in the response to inositol. For example, if Ino2 levels are extremely low under repressing conditions, then the cell would have to express *INO2* prior to activating transcription of the target genes. Alternatively, the "pump may be primed" by a small amount of Ino2 and derepression of *INO2* expression may serve to establish the degree of derepression of the target genes. We favor the latter model, since it has been shown that extracts prepared from cells grown under repressing conditions form the Ino2/Ino4/UAS<sub>INO</sub> complex (32). Furthermore, the kinetics of derepression of an *INO2-cat* gene and an *INO1-cat* gene were essentially identical (23), suggesting that derepression of *INO2* expression does not precede that of its target genes.

The role of derepressing *INO2* expression may be to establish the degree of derepression of the target genes. Consistent with this hypothesis, we have observed a correlation between *INO2* expression and target gene expression at different concentrations of inositol (23). Thus, depending on the inositol concentration, *INO2* may be expressed at different levels which will determine the level of target gene expression. Moreover, since the number and sequence of potential Ino2/Ino4 target sequences vary among the promoters of the coregulated genes (15), it seems likely that different levels of *INO2* expression may be required to activate expression of different target genes.

The experiments presented here provide further evidence that *INO2* expression is limiting relative to *INO4* (23) and that target gene expression is most likely limited by the amount of *INO4* expression. This latter point is evidenced by the fact that *INO2* expression from the *GAL1* promoter increased linearly as a function of galactose concentration up to 2% galactose (Fig. 3B), whereas *INO1* and *CHO1* expression reached a plateau at galactose concentrations between 0.25% and 0.5%. This result was not entirely surprising, since we previously observed that the *INO2* promoter was substantially weaker than the *INO4* promoter (23). Furthermore, overexpression of *INO2* (but not *INO4*) from a multicopy plasmid yielded an elevated level of the Ino2/Ino4/UAS<sub>INO</sub> complex in mobility-shift assays (20). Thus, the role of the *INO4* gene product may be to establish an upper limit to the level of derepression of the target genes.

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- Guarente, L. (1987) *Annu. Rev. Genet.* **21**, 425–452.
- Struhl, K. (1989) *Annu. Rev. Biochem.* **58**, 1051–1077.
- Johnston, M. (1987) *Microbiol. Rev.* **51**, 458–476.
- Hinnebusch, A. G. (1988) *Microbiol. Rev.* **52**, 248–273.
- Herskowitz, I. (1989) *Nature (London)* **342**, 749–757.
- Guarente, L. (1988) *Cell* **52**, 303–305.
- Griggs, D. W. & Johnston, M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8597–8601.
- Hinnebusch, A. G. (1988) *Trends Genet.* **4**, 169–174.
- Kornitzer, D., Raboy, B., Kulka, R. G. & Fink, G. R. (1994) *EMBO J.* **13**, 6021–6030.
- Nasmyth, K., Adolf, G., Lydall, D. & Seddon, A. (1990) *Cell* **62**, 631–647.
- Dohrmann, P. R., Butler, G., Tamai, K., Dorland, S., Greene, J. R., Thiele, D. J. & Stillman, D. J. (1992) *Genes Dev.* **6**, 93–104.
- Leuther, K. K. & Johnston, S. A. (1992) *Nature (London)* **256**, 1333–1335.
- Jayaraman, P.-S., Hirst, K. & Goding, C. R. (1994) *EMBO J.* **13**, 2192–2199.
- Nikoloff, D. M. & Henry, S. A. (1991) *Annu. Rev. Genet.* **25**, 559–583.
- Paultauf, F., Kohlwein, S. D. & Henry, S. A. (1992) in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*, eds. Jones, E. W., Pringle, J. R. & Broach, J. R. (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. 2, pp. 415–500.
- Lopes, J. M., Hirsch, J. P., Chorgo, P. A., Schulze, K. L. & Henry, S. A. (1991) *Nucleic Acids Res.* **19**, 1687–1693.
- Bailis, A. M., Lopes, J. M., Kohlwein, S. D. & Henry, S. A. (1992) *Nucleic Acids Res.* **20**, 1411–1418.
- Blackwell, T. K. & Weintraub, H. (1990) *Science* **250**, 1104–1110.
- Blackwell, T. K., Huang, J., Averil, M., Kretzner, L., Alt, F. A., Eisenman, R. N. & Weintraub, H. (1993) *Mol. Cell. Biol.* **13**, 5216–5224.
- Nikoloff, D. M. & Henry, S. A. (1994) *J. Biol. Chem.* **269**, 7402–7411.
- Ambroziak, J. & Henry, S. A. (1994) *J. Biol. Chem.* **269**, 15344–15349.
- Schwank, S., Ebbert, R., Rautenstrauss, K., Schweizer, E. & Schüller, H.-J. (1995) *Nucleic Acids Res.* **23**, 230–237.
- Ashburner, B. P. & Lopes, J. M. (1995) *Mol. Cell. Biol.* **15**, 1709–1715.
- White, M. J., Hirsch, J. P. & Henry, S. A. (1991) *J. Biol. Chem.* **266**, 863–872.
- Hirsch, J. P. & Henry, S. A. (1986) *Mol. Cell. Biol.* **6**, 3320–3328.
- Griggs, D. W. & Johnston, M. (1993) *Mol. Cell. Biol.* **13**, 4999–5009.
- Elion, E. A. & Warner, J. R. (1984) *Cell* **39**, 663–673.
- Bailis, A., Poole, M., Carman, G. & Henry, S. (1987) *Mol. Cell. Biol.* **7**, 167–176.
- Aparicio, O. M. & Gottschling, D. E. (1994) *Genes Dev.* **8**, 1133–1146.
- Shimada, H. & Fukusawa, T. (1985) *Gene* **39**, 1–9.
- Nogi, Y., Shimada, H., Matsuzaki, Y., Hashimoto, H. & Fukusawa, T. (1984) *Mol. Gen. Genet.* **195**, 29–34.
- Lopes, J. M. & Henry, S. A. (1991) *Nucleic Acids Res.* **19**, 3987–3994.