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# Genome Expression Analysis in Yeast Reveals Novel Transcriptional Regulation by Inositol and Choline and New Regulatory Functions for Opi1p, Ino2p, and Ino4p*

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**Teresa C. Santiago and Choukri Ben Mamoun‡**

*From the Center for Microbial Pathogenesis and the Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, Connecticut 06030-3710*

**In *Saccharomyces cerevisiae*, genes encoding phospholipid-synthesizing enzymes are regulated by inositol and choline (IC). The current model suggests that when these precursors become limiting, the transcriptional complex Ino2p-Ino4p activates the expression of these genes, whereas repression requires Opi1p and occurs when IC are available. In this study, microarray-based expression analysis was performed to assess the global transcriptional response to IC in a wild-type strain and in the *opi1*Δ, *ino2*Δ, and *ino4*Δ null mutant strains. Fifty genes were either activated or repressed by IC in the wild-type strain, including three already known IC-repressed genes. We demonstrated that the IC response was not limited to genes involved in membrane biogenesis, but encompassed various metabolic pathways such as biotin synthesis, one-carbon compound metabolism, nitrogen-containing compound transport and degradation, cell wall organization and biogenesis, and acetyl-CoA metabolism. The expression of a large number of IC-regulated genes did not change in the *opi1*Δ, *ino2*Δ, and *ino4*Δ strains, thus implicating new regulatory elements in the IC response. Our studies revealed that Opi1p, Ino2p, and Ino4p have dual regulatory activities, acting in both positive and negative transcriptional regulation of a large number of genes, most of which are not regulated by IC and only a subset of which is involved in membrane biogenesis. These data provide the first global response profile of yeast to IC and reveal novel regulatory mechanisms by these precursors.**

The soluble precursors inositol and choline (IC)[1] exert a major regulatory effect on the enzymes of the phospholipid, sterol, and fatty acid biosynthetic pathways (1). The available data suggest the following model for IC-mediated regulation of gene expression (1, 2). When extracellular levels of these precursors become limiting, a transcriptional heterodimeric complex composed of the basic helix-loop-helix proteins, Ino2p, and Ino4p (3, 4) binds to a conserved *cis*-acting upstream activating sequence designated the IC-responsive element, also known as $UAS_{INO}$, which resides in the promoters of many genes encoding phospholipid, fatty acid, and sterol biosynthetic enzymes and activates their transcription (5–9). In the presence of IC, expression of these genes is down-regulated. Several genes encoding enzymes involved in lipid metabolism have been shown to exhibit this pattern of regulation. However, the repression ratios vary from one gene to another. *INO1*, which encodes the inositol-1-phosphate synthase, is the most highly repressed gene with at least 30-fold repression in response to IC (10). Other genes such as those encoding phosphatidylserine synthase (*PSS1*/*CHO1*), CDP-diacylglycerol synthase (*CDS1*), phosphatidylcholine decarboxylase (*PSD1*), phospholipid methyltransferases (*CHO2*/*PEM1* and *OPI3*/*PEM2*), choline kinase (*CKI1*), choline phosphotransferase (*CPT1*), the α-subunit of the fatty-acid synthase (*FAS1*), the inositol transporter (*ITR1*), phosphatidylglycerophosphate synthase (*PGS1*), and acetyl-CoA carboxylase (*ACC1*) exhibit lower expression ratios (1, 11–22). IC-mediated repression of *INO1* also requires a functional *OPI1* gene, which encodes the Opi1p protein, postulated to act as a repressor of the transcription of the $UAS_{INO}$-containing genes (23). Disruption of *OPI1* results in a complete loss of IC-mediated repression of *INO1*. Overexpression of *OPI1* results in inhibition of activation of expression of $UAS_{INO}$-containing genes even in the absence of IC (23) and renders wild-type yeast auxotrophic for inositol, supporting the idea that it functions as a negative regulator of phospholipid biosynthesis. Interestingly, the expression of both *INO2* and *OPI1* genes is also down-regulated in the presence of IC (24, 25). Conversely, the activity and/or transcription of genes encoding diacylglycerol-pyrophosphate phosphatase (*DPP1*), inositol-phosphorylceramide synthase (*AUR1*), one form of $Mg^{2+}$-dependent phosphatidate phosphatase, and *myo*-inositol 1-monophosphatase (*INM1*) has been reported to be moderately up-regulated by inositol (26–29). However, the mechanism of inositol-mediated transcriptional activation has not been investigated. The regulation of gene expression by IC suggests the presence of a specialized transduction pathway. However, little is known about the possible components of this pathway. Ino2p, the main player in the heterodimeric complex, is suggested to be the target of such a signaling pathway, as the overexpression of *INO2* (but not of *INO4*) counteracts the repression mediated by IC (30, 31).

In this study, we analyzed global gene expression in response to IC in wild-type, *opi1*Δ, *ino2*Δ, and *ino4*Δ yeast strains. We found 50 genes that were either repressed or activated by at least 3-fold in response to IC in the wild-type strain, including three known IC-repressed genes. IC regulation affected various metabolic pathways, including biotin synthesis, one-carbon compound metabolism and methionine synthesis, nitrogen

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‡ To whom correspondence should be addressed: Center for Microbial Pathogenesis, E7041, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06030-3710. Tel.: 860-679-3544; Fax: 860-679-8130; E-mail: choukri@up.uchc.edu.

[1] The abbreviations used are: IC, inositol and choline; RT, reverse transcription.

TABLE I
*Oligonucleotides used in this study*

| Gene name | 5′-Oligonucleotide | 3′-Oligonucleotide |
|---|---|---|
| *ACT1* | 5′-CGGTATGGGTCAAAAAGACTCCTAC-3′ | 5′-GGACAAAACGGCTTGGATGG-3′ |
| *BIO2* | 5′-AGTCATTCGTGGACAAAATCGC-3′ | 5′-TCGTCTTCGCTTTCACCGAG-3′ |
| *BIO3* | 5′-TGGTAGAACAGGTGAGATTTTCGC-3′ | 5′-TGGTGGCATAATGTAACACAGGC-3′ |
| *BIO4* | 5′-CGCATCAACTTGGCAACCAC-3′ | 5′-TGACACCGAACTTCTTCAGGGC-3′ |
| *BIO5* | 5′-TACGAGGACCCTTCTGACGATG-3′ | 5′-AACCGACAACAAACGCCATTC-3′ |
| *GCV1* | 5′-GAGACACCGAGTTCTTTCACGATG-3′ | 5′-GCTGGTCCATTATCTTGGCATAGC-3′ |
| *INO1* | 5′-GGCAACAATGGCTCCACTTTAG-3′ | 5′-GAGGCTTCACCAAGGACATCTTC-3′ |
| *MEP2* | 5′-GGACTACAGTTGGTTTGTGTTCAGG-3′ | 5′-TGAGGAGGTGGCATTTGTGC-3′ |
| *MTD1* | 5′-CTAACGGCGATGACTCTGTGAAC-3′ | 5′-CCTTCTTTGATGTATTCGGTGGG-3′ |
| *OPI3* | 5′-TCCAGCAACTCATCCACAGTGTC-3′ | 5′-TGCCCCAAACCAAAGAGAGC-3′ |
| *OPT1* | 5′-GGAAGCAATGACTAACCAACACG-3′ | 5′-TCAAGAAGAACCACATCAGCGG-3′ |
| *SAM2* | 5′-TCCATTCTCCAAGGTTGCCTG-3′ | 5′-CCCATCTACCATTGTCGTCTTCG-3′ |
| *VHT1* | 5′-CTCAAACCAAGGCTGAAAGACG-3′ | 5′-AAGTCCTGAAGTCACGGAACCC-3′ |

transport and degradation, cell wall organization and biogenesis, and acetyl-CoA metabolism. The function of 13 genes is unknown. These data, which provide the first global response of yeast to IC, reveal novel regulatory mechanisms by these precursors and indicate that Opi1p, Ino2p, and Ino4p are involved in both positive and negative regulation of expression of a large number of genes, only a subset of which is regulated by IC and is involved in phospholipid biosynthesis.

## EXPERIMENTAL PROCEDURES

*Strains and Media—Saccharomyces cerevisiae* strains BY4741 (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), *opi1*Δ (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 opi1*::*KAN*$^r$), *ino2*Δ (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ino2*::*KAN*$^r$), and *ino4*Δ (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ino4*::*KAN*$^r$) were used in this study. The growth media used in this study were minimal medium without inositol ($SDI_0$: 1.7% yeast nitrogen base lacking inositol, 5% ammonium sulfate, and 2% dextrose) and minimal medium with IC ($SDI_{50}$: 1.7% yeast nitrogen base lacking inositol, 5% ammonium sulfate, 2% dextrose, 50 μM inositol, and 1 mM choline). Appropriate amino acids (200 mg/liter each) were added to maintain cell growth.

*Growth Conditions and RNA Preparation*—Wild-type and *opi1*Δ strains were grown overnight in $SDI_0$, after which they were diluted and grown in $SDI_{50}$ at 30 °C to an absorbance of 0.7 at 600 nm. The *ino2*Δ and *ino4*Δ strains were cultivated overnight in $SDI_{50}$, diluted, and grown in $SDI_{50}$ at 30 °C to $A_{600}$ = 0.7. Duplicates were prepared for each condition. RNA was obtained as described by Schmitt *et al.* (32). Harvested cells were resuspended in 50 mM sodium acetate (pH 5.3) and 10 mM EDTA and then treated with SDS and acidic phenol. After vigorous vortexing, the cells were incubated at 65 °C for 4 min and chilled in a dry ice/ethanol mixture. Suspensions were centrifuged, and the aqueous layer was collected, acidified with ammonium acetate, and precipitated with absolute ethanol at −20 °C. Total RNA was purified using the RNeasy RNA purification kit (QIAGEN Inc.).

*Microarray Analysis*—Preparation of cRNA and hybridizations were performed at the HHMI Biopolymer-Keck Foundation Biotechnology Resource Laboratory. The GeneChip yeast genome S98 array from Affymetrix was used in all hybridizations. The array contains ~6300 annotated genes from *S. cerevisiae*. "Comparison expression analyses" were performed using Affymetrix MAS Version 5.0 software as recommended by Affymetrix. Four pairwise comparisons (expressed as signal log ratios) were obtained between data sets ($n$ = 2) from the experiment and data sets ($n$ = 2) from the base line. For each gene, the average of the four signal log ratios was computed and converted to a -fold change value.[2] Clustering analysis was performed using the Spotfire program.

*Reverse Transcription (RT)-PCR*—Total RNA was treated with DNase I (Promega) for 30 min at 37 °C. First-strand cDNA synthesis was performed using the SuperScript first-strand synthesis system for RT-PCR (Invitrogen). Three μg of DNase-treated RNA, 3 μl of random hexamers (50 ng/μl), and 1 μl of dNTPs (10 mM) were combined and adjusted to 10 μl with diethyl pyrocarbonate-treated water. The mixture was then incubated at 65 °C for 5 min and chilled. The RNA/primer mixture, RT buffer, $MgCl_2$ (5 mM final concentration), dithiothreitol (0.01 M final concentration), and ribonuclease inhibitor were added to a final volume of 19 μl. After incubation at 25 °C for 2 min, 50 units (1 μl) of SuperScript II RT was added, and the mixture was incubated at 25 °C for 10 min, 42 °C for 50 min, and 70 °C for 15 min and then chilled briefly. The mixture was treated with RNase H at 37 °C for 20 min. PCR was performed to amplify the *BIO2*, *BIO3*, *BIO4*, *BIO5*, *VHT1*, *MEP2*, *INO1*, *OPI3*, *SAM2*, *GCV1*, *MTD1*, *OPT1*, and *ACT1* genes using a 87-fold diluted sample of the cDNA as template and titanium *Taq* polymerase (Clontech). The following PCR conditions were used: 94 °C for 2 min; 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 45 s; and finally, 68 °C for 3 min. The primers used in this study are listed in Table I.

*Real-time PCR*—The LightCycler-FastStart DNA Master SYBR Green I system (Roche Applied Science) was used to amplify the *INO1*, *BIO5*, *OPT1*, and *ACT1* genes according to the manufacturer's instructions, using a 24-fold diluted sample of the cDNA as template and the primers described above. Preincubation was done at 95 °C for 10 min, followed by amplification for 45 cycles under the following conditions: 95 °C for 10 s, 55 °C for 5 s, and 72 °C for 20 s. For each gene, the -fold change between the experiment and the base line was calculated as a log value of the difference between the crossing points of the PCRs. The average -fold change is computed from duplicate samples.

## RESULTS

*Whole Genome Survey for IC-regulated Genes*—We have applied microarray analysis to study the global response profile of yeast cells to the presence of the phospholipid precursors inositol and choline. A cDNA probe prepared from poly(A)$^+$ RNA isolated from a wild-type yeast strain (BY4741) cultivated in minimal medium lacking or supplemented with IC ($IC_{50}$) [3] was used to hybridize the GeneChip yeast genome S98 array from Affymetrix. The transcript levels of 50 annotated protein-encoding genes changed at least 3-fold in response to $IC_{50}$ (Table II). Of those, 39 were repressed, whereas 11 were activated. Only 10 (*INO1*, *OPI3*, *PSD1*, *VHT1*, *SAM2*, *SAG1*, *ACH1*, *SRO77*, YEL073C, and YJR008W) of the 39 IC-repressed genes contain at least one copy of the $UAS_{INO}$ sequence within their promoter regions (Table II). Of the 50 $IC_{50}$-regulated genes, only three, *INO1*, *OPI3*, and *PSD1*, which play an important role in membrane biogenesis, were previously known to be regulated by IC. As expected, these three genes were repressed under $IC_{50}$ conditions. *INO1* showed the highest level of repression (46.9-fold). Of the 50 $IC_{50}$-regulated genes, 37 have known or predicted cellular functions, and the remaining 13 encode proteins of unknown function (YEL073C, YJR008W, YDL038C, YDL039C, YBR056w-a, YGR213C, YGR161C, YLR136C, YER078C, YJL048C, YDL241W, YLR413W, and YKR075C). Of the 11 $IC_{50}$-activated genes, eight (*GCV1*, *MMP1*, *OPT1*, *MTD1*, *CWP1*, *ECM13*, *MSC2*, and *NDE1*) encode proteins with known and predicted functions, and three (YDL241W, YLR413W, and YKR075C) have unknown function. None of these genes was previously known to be IC-regulated or to harbor a $UAS_{INO}$ sequence in the promoter region.

[2] The complete data sets are available upon request.

[3] IC50 is used to highlight the concentration of inositol (50 μM) used in this study.

TABLE II

*Classification into functional groups of genes whose transcripts are repressed or induced by at least 3-fold by $IC_{50}$ in the wild-type strain*

| ORF[a] code | Gene name | Fold change: WT + $IC_{50}$ *vs* WT − IC | Fold change: *opi1*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino2*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino4*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Gene description |
|---|---|---|---|---|---|---|
| Phospholipid biosynthesis and transport | | | | | | |
| YJL153C[b] | *INO1* | −46.9 | 56.7 | −1.2 | −2.1 | Inositol-1-phosphate synthase |
| YCR098C | *GIT1* | −6.5 | −1.3 | −1.2 | −1.5 | Phospholipid transporter, general substrate transporter |
| YJR073C[b] | *OPI3* | −6.1 | 7.2 | −2.4 | −2.5 | Methylene-fatty-acyl-phospholipid synthase |
| YNL169C[b] | *PSD1* | −3.0 | 4.3 | 1.6 | 1.4 | Phosphatidylserine decarboxylase |
| Biotin synthesis and transport | | | | | | |
| YNR056C | *BIO5* | −40.1 | −1.4 | −1.8 | −1.4 | KAPA permease |
| YNR058W | *BIO3* | −23.8 | −1.1 | −1.1 | −1.0 | Adenosylmethionine-8-amino-7-oxononanoate aminotransferase |
| YNR057C | *BIO4* | −6.4 | 1.0 | −1.1 | −1.1 | Dethiobiotin synthase |
| YGR286C | *BIO2* | −6.1 | 1.1 | −1.1 | 1.1 | Biotin synthase |
| YGR065C[b] | *VHT1* | −3.9 | 1.2 | −1.1 | 1.0 | Biotin transporter |
| One-carbon compound metabolism and methionine synthesis | | | | | | |
| YDR502C[b] | *SAM2* | −6.8 | 7.3 | −1.2 | −1.4 | S-Adenosylmethionine transferase |
| YGL125W | *MET13* | −3.1 | 2.0 | −1.5 | −1.1 | Methylenetetrahydrofolate reductase |
| YDR019C | *GCV1*[d] | 4.8 | −1.9 | −1.4 | −1.1 | Glycine dehydrogenase, aminomethyltransferase |
| YLL061W | *MMP1*[d] | 4.3 | −2.8 | 2.0 | 1.6 | *S*-Methylmethionine transporter |
| YJL212C | *OPT1*[d] | 4.2 | −2.5 | 1.3 | −1.4 | Glutathione transporter |
| YKR080W | *MTD1*[d] | 3.2 | −1.6 | −1.5 | −1.2 | Methylenetetrahydrofolate dehydrogenase |
| Nitrogen transport and degradation | | | | | | |
| YGR138C | *TPO2* | −6.0 | 3.9 | 6.3 | 5.5 | Spermine transporter, general substrate transporter |
| YBR006W | *UGA2* | −4.8 | 3.0 | 1.5 | 1.5 | Succinate-semialdehyde dehydrogenase |
| YNL142W | *MEP2* | −4.3 | −1.1 | −1.3 | 1.0 | Ammonium transporter |
| YHL016C | *DUR3* | −3.9 | 1.4 | 2.0 | 2.1 | Urea transporter, $Na^+$/solute symporter |
| YOR273C | *TPO4* | −3.0 | 2.5 | −1.1 | −1.0 | Spermidine transporter, spermine transporter |
| Cell wall organization and biogenesis | | | | | | |
| YJR004C[b] | *SAG1* | −3.7 | 1.2 | 1.0 | 1.3 | Cell adhesion receptor |
| YJR150C | *DAN1* | −3.4 | 2.8 | [c] | 3.5 | Structural component of cell wall, sterol transport |
| YKL096W | *CWP1*[d] | 3.4 | 1.1 | −1.2 | −1.1 | Structural constituent of cell wall |
| YBL043W | *ECM13*[d] | 3.0 | −1.4 | −1.4 | −1.5 | Cell wall organization and biogenesis |
| Acetyl-CoA metabolism | | | | | | |
| YPR001W | *CIT3* | −4.2 | 1.1 | −1.9 | −1.1 | Citrate synthase |
| YBL015W[b] | *ACH1* | −4.1 | 1.1 | 1.2 | 1.1 | Acetyl-CoA hydrolase/transferase |
| YOR100C | *CRC1* | −3.6 | −2.0 | −1.7 | −1.5 | Camitine/acyl camitine carrier |
| Unrelated pathways | | | | | | |
| YJR155W | *AAD10* | −9.5 | −1.1 | −1.1 | −1.1 | Benzyl-alcohol dehydrogenase |
| YMR271C | *URA10* | −9.0 | 4.3 | 1.0 | 1.1 | Orotate phosphoribosyltransferase |
| YOR328W | *PDR10* | −5.6 | −1.2 | 1.0 | 1.2 | ATP-binding cassette transporter |
| YBR093C | *PHO5* | −5.3 | 1.3 | −1.3 | −1.3 | Acid phosphatase |
| YBL106C[b] | *SRO77* | −4.5 | 5.3 | −1.0 | −1.1 | Golgi-to-plasma membrane transport |
| YGL156W | *AMS1* | −3.9 | 1.9 | 2.8 | 3.2 | α-Mannosidase |
| YJR047C | *ANB1* | −3.1 | 1.4 | 1.1 | −1.0 | Translation initiation factor |
| YNL333W | *SNZ2* | −3.0 | 1.3 | 1.3 | 1.1 | Vitamin $B_6$ biosynthesis protein |
| YDR205W | *MSC2*[d] | 3.5 | −6.2 | 1.1 | −1.0 | Cation transporter |
| YMR145C | *NDE1*[d] | 3.0 | −1.4 | 1.3 | 1.0 | NADH dehydrogenase, disulfide oxidoreductase |
| Unknown | | | | | | |
| YEL073C[b] | | −32.6 | 34.3 | 1.2 | −1.7 | |
| YJR008W[b] | | −4.7 | 5.9 | 1.2 | −1.1 | |
| YDL038C | | −4.4 | 1.1 | 1.7 | −1.1 | |
| YDL039C | *PRM7* | −4.0 | 1.2 | 1.7 | −1.1 | |
| YBR056w-a | | −3.8 | −1.1 | 1.8 | 1.6 | |
| YGR213C | *RTA1* | −3.7 | −1.2 | 1.5 | 1.6 | |
| YGR161C | *RTS3* | −3.4 | 1.2 | 1.6 | 1.5 | |
| YLR136C | *TIS11* | −3.2 | 1.1 | 1.1 | −1.4 | |
| YER078C | | −3.2 | 2.4 | 1.1 | −1.1 | |
| YJL048C | | −3.0 | 2.7 | 1.3 | 1.1 | |
| YDL241W[d] | | 3.7 | −1.7 | −2.1 | −2.1 | |
| YLR413W[d] | | 3.4 | −1.7 | 1.2 | 1.5 | |
| YKR075C[d] | | 3.1 | −1.8 | 1.7 | −1.2 | |

[a] ORF, open reading frame; WT, wild-type; KAPA, 7-keto-8-aminopelargonic acid.
[b] $UAS_{INO}$ present within the 500-bp upstream region.
[c] Undetermined due to significant S.D. in duplicate samples.
[d] $IC_{50}$-activated genes.

*Cellular Functions Regulated by $IC_{50}$*—All known yeast genes encoding biotin-synthesizing enzymes as well as transporters of biotin and its precursor were repressed in $IC_{50}$ (Table II). The most highly repressed genes of this group were *BIO5* and *BIO3*, with 40- and 24-fold repression ratios. *BIO5* encodes a permease that is involved in the transport of the biotin precursor 7-keto-8-aminopelargonic acid, and *BIO3* encodes an enzyme that catalyzes the conversion of this precursor into

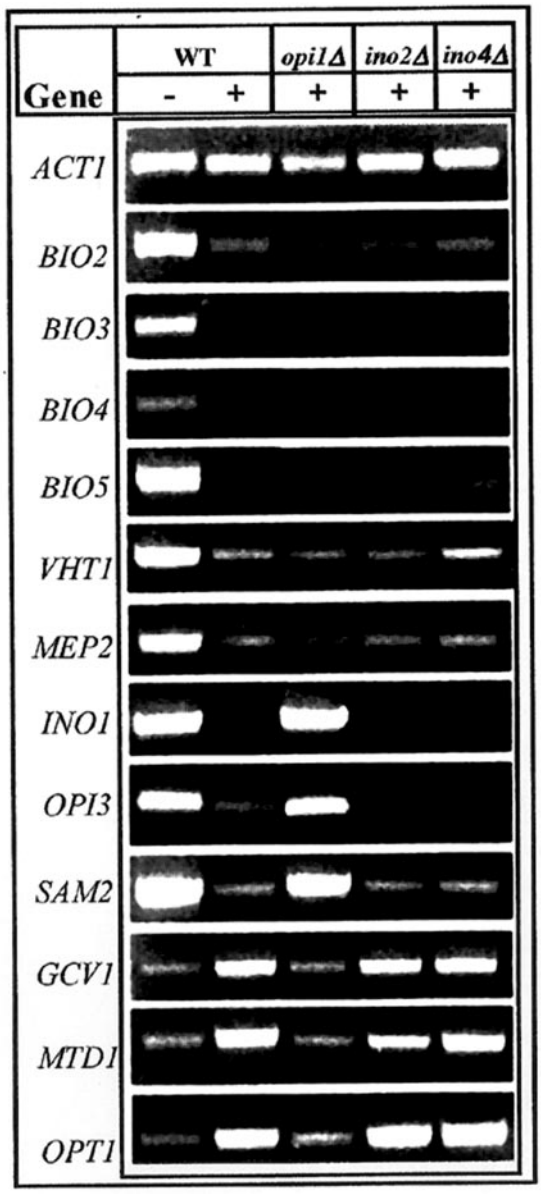


FIG. 1. **Semiquantitative RT-PCR analysis of genes activated or repressed by $IC_{50}$.** The expression of *BIO2*, *BIO3*, *BIO4*, *BIO5*, *VHT1*, *MEP2*, *INO1*, *OPI3*, *SAM2*, *GCV1*, *MTD1*, and *OPT1* in the wild-type (*WT*), *opi1*Δ, *ino2*Δ, and *ino4*Δ strains grown in the presence (+) or absence (−) of $IC_{50}$ was analyzed as described under "Experimental Procedures." *ACT1* was used as a control.

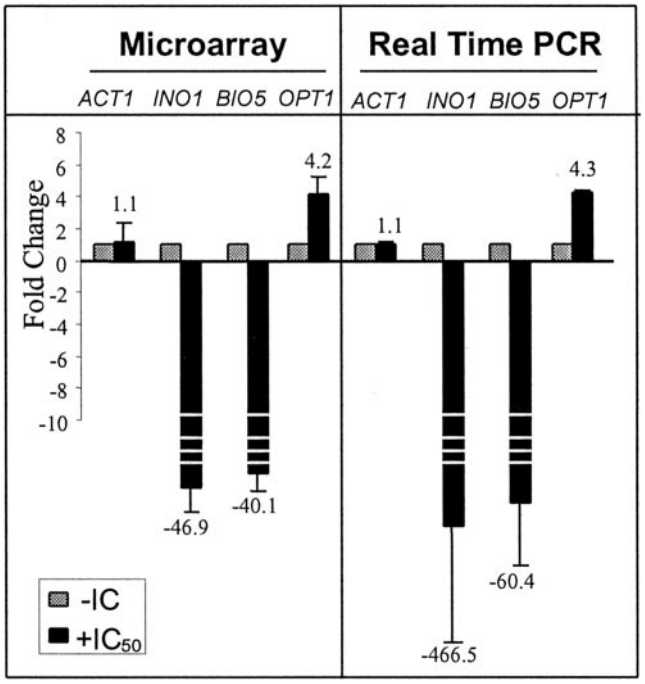


FIG. 2. **Comparative microarray data and real-time PCR analyses of gene repression and activation by $IC_{50}$.** The expression of *INO1*, *BIO5*, and *OPT1* was analyzed in the wild-type strain grown in the absence (*gray bars*) or presence (*black bars*) of $IC_{50}$ as described under "Experimental Procedures." -Fold repression or activation is indicated accordingly. *ACT1* was used as a control.

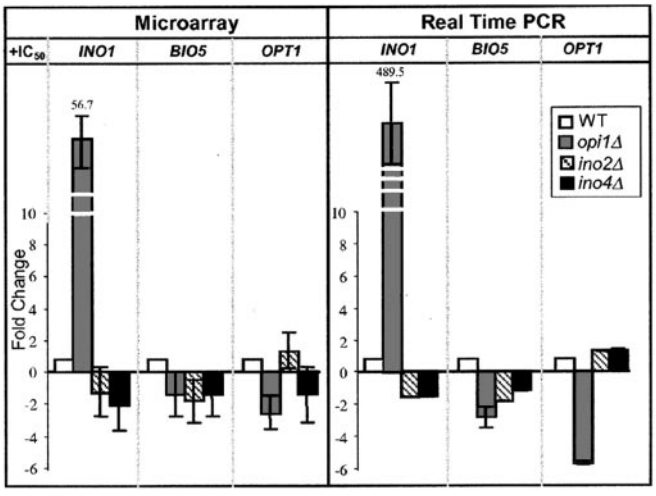


FIG. 3. **Comparative microarray data and real-time PCR analyses of gene repression and activation in the *opi1*Δ, *ino2*Δ, and *ino4*Δ strains relative to the wild-type strain.** The level of expression in the presence of $IC_{50}$ of *INO1*, *BIO5*, and *OPT1* in the *opi1*Δ (*gray bars*), *ino2*Δ (*hatched bars*), and *ino4*Δ (*black bars*) strains was compared to that in the wild-type (*WT*, *white bars*) as described under "Experimental Procedures."

7,8-diaminopelargonic acid. *BIO4*, *BIO2*, and *VHT1*, which were repressed by 6-, 6-, and 4-fold, respectively, encode proteins involved in the catalysis of dethiobiotin synthesis from 7,8-diaminopelargonic acid, synthesis of biotin from dethiobiotin, and high affinity transport of biotin, respectively. These results thus reveal an important regulatory role for the phospholipid precursors in biotin biosynthesis.

Metabolism of one-carbon compounds, which are derived from the catabolism of serine, glycine, and formate (33–37) and are important for the synthesis of methionine and purines, was also affected by $IC_{50}$. *GCV1*, encoding glycine dehydrogenase, a component of a protein complex involved in glycine catabolism in mitochondria (38), was induced by ~5-fold in $IC_{50}$. The expression of *MTD1*, encoding NAD-dependent tetrahydrofolate dehydrogenase, was induced by ~3-fold, whereas the expression of *MET13*, encoding tetrahydrofolate reductase, was repressed by ~3-fold. *SAM2*, encoding *S*-adenosylmethionine transferase, was repressed by ~7-fold, whereas *OPT1* and *MMP1*, encoding the glutathione and *S*-methylmethionine transporters, respectively, were activated by ~4-fold in the wild-type strain.

Several genes that encode transporters of nitrogen-containing compounds were repressed in $IC_{50}$. *MEP2* and *DUR3*, involved in the transport of ammonium and urea, respectively, were both repressed by ~4-fold. *TPO2* and *TPO4*, encoding polyamine transporters localized in both plasma and vacuolar membranes, were repressed by 6- and 3-fold, respectively. Finally, *UGA2*, involved in the degradation of 4-aminobutyrate, was also repressed by ~5-fold. Four genes, *DAN1*, *CWP1*,

TABLE III
*Genes that require Opi1p, Ino2p, and Ino4p for regulation in the presence of $IC_{50}$ based on the 3-fold cutoff*

| ORF[a] code | Gene name | Fold change: WT + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *opi1*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino2*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino4*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Gene description |
|---|---|---|---|---|---|---|
| Genes that require Opl1p, Ino2p, and Ino4p for negative regulation | | | | | | |
| YGR138C | *TPO2* | 1.0 | 3.9 | 6.3 | 5.5 | Spermine transporter |
| YPR160W | *GPH1* | 1.0 | 3.8 | 4.6 | 5.2 | Glycogen phosphorylase |
| YFR053C | *HXK1* | 1.0 | 3.4 | 9.4 | 6.5 | Hexokinase |

[a] ORF, open reading frame; WT, wild-type.

TABLE IV
*Genes that require Opi1p, but have little or no dependence on Ino2p and Ino4p for regulation in $IC_{50}$ based on the 3-fold cutoff*

| ORF[a] code | Gene name | Fold change: WT + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *opi1*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino2*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino4*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Gene description |
|---|---|---|---|---|---|---|
| Genes that require Opi1p for negative regulation | | | | | | |
| YJL153C | *INO1* | 1.0 | 56.7 | −1.2 | −2.1 | Inositol-1-phosphate synthase |
| YEL073C | | 1.0 | 34.3 | 1.2 | −1.7 | Unknown |
| YDR502C | *SAM2* | 1.0 | 7.3 | −1.2 | −1.4 | *S*-Adenosylmethionine transferase |
| YJR073C | *OPI3* | 1.0 | 7.2 | −2.4 | −2.5 | Methylene-fatty-acyl-phospholipid synthase |
| YJR008W | | 1.0 | 5.9 | 1.2 | −1.1 | Unknown |
| YBR006W | *UGA2* | 1.0 | 5.4 | 1.3 | −1.1 | Succinate-semialdehyde dehydrogenase |
| YBL106C | *SRO77* | 1.0 | 5.3 | −1.0 | −1.1 | Golgi-to-plasma membrane transport |
| YMR271C | *URA10* | 1.0 | 4.3 | 1.0 | 1.1 | Orotate phosphoribosyltransferase |
| YNL169C | *PSD1* | 1.0 | 4.3 | 1.6 | 1.4 | Phosphatidylserine decarboxylase |
| YER026C | *CHO1* | 1.0 | 3.7 | 1.3 | 1.4 | CDP-diacylglycerol: serine *O*-phosphatidyltransferase |
| YHR067W | *RMD12* | 1.0 | 3.7 | 1.4 | 1.2 | Unknown |
| YDL049C | *KNH1* | 1.0 | 3.2 | 1.3 | 1.0 | β-1,6-Glucan biosynthesis |
| YDR497C | *ITR1* | 1.0 | 3.1 | −1.2 | −1.1 | *myo*-Inositol transporter |
| YBR177C | *EHT1* | 1.0 | 3.0 | 1.1 | 1.1 | Lipid metabolism |
| Gene that requires Opi1p for positive regulation | | | | | | |
| YDR205W | *MSC2* | 1.0 | −6.2 | 1.1 | −1.0 | Cation transporter |

[a] ORF, open reading frame; WT, wild-type.

TABLE V
*Genes that require Ino2p, but have little or no dependence on Opi1p and Ino4p for regulation in the presence of $IC_{50}$ based on the 3-fold cutoff*

| ORF[a] code | Gene name | Fold change: WT + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *opi1*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino2*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino4*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Gene description |
|---|---|---|---|---|---|---|
| Genes that require Ino2p for negative regulation | | | | | | |
| YLR327C | | 1.0 | 1.1 | 3.9 | 2.8 | Unknown |
| YDR277C | *MTH1* | 1.0 | 1.0 | 3.5 | 1.9 | Signal transduction, glucose transport |
| YJL116C | *NCA3* | 1.0 | 1.5 | 3.3 | 2.5 | Mitochondrion organization and biogenesis |
| YDR461W | *MFA1* | 1.0 | 2.5 | 3.2 | 2.9 | Pheromone |
| YER150W | *SPI1* | 1.0 | 1.6 | 3.2 | 2.6 | Unknown |
| YDR343C | *HXT6* | 1.0 | 1.6 | 3.1 | 2.7 | Fructose, glucose, mannose transporter |
| YGR008C | *STF2* | 1.0 | 2.2 | 3.0 | 2.5 | ATP synthesis-coupled proton transport |

[a] ORF, open reading frame; WT, wild-type.

*SAG1*, and *ECM13*, encoding proteins known or predicted to function in cell wall biogenesis were also regulated by $IC_{50}$. *DAN1* and *SAG1*, which encode a cell wall mannoprotein and a cell adhesion receptor, respectively, were repressed by 3- and 4-fold, respectively, whereas *CWP1* and *ECM13*, which encode a cell mannoprotein and a protein involved in hypersensitivity to the cell-surface polymer-perturbing agent calcofluor white, respectively, were induced by ~3-fold. Finally, three genes involved in acetyl-CoA metabolism, *ACH1*, *CRC1* and *CIT3*, encoding acetyl-CoA hydrolase, acyl-carnitine carrier, and citrate synthase activities, respectively, were also repressed by ~4-fold in response to $IC_{50}$.

*Role of Opi1p in $IC_{50}$-mediated Transcriptional Regulation*—Opi1p was previously shown to be required for repression of *INO1* and other phospholipid genes regulated in the presence of IC (23, 39, 40). To examine the role of Opi1p in the $IC_{50}$ response, we examined the expression profile of the 50 $IC_{50}$-regulated genes in the *opi1*Δ mutant background. Of the 39 $IC_{50}$-repressed genes, only 10 were derepressed by >3-fold when Opi1p function was lost (Table II). As expected, repression of the phospholipid genes *INO1*, *OPI3*, and *PSD1* in $IC_{50}$ was shown to be highly dependent on Opi1p, with 56.7-, 7.2-, and 4.3-fold derepression with the loss of Opi1p function, respectively (Tables II and IV). The seven other Opi1p-dependent $IC_{50}$-repressed genes (*SAM2*, *TPO2*, *UGA2*, *URA10*, *SRO77*, YEL073C, and YJR008W) are involved in various metabolic pathways. Four $IC_{50}$-repressed genes, *TPO4*, *DAN1*, YER078C, YJL048C, were moderately affected by the loss of Opi1p function, exhibiting >2-fold derepression in *opi1*Δ, suggesting that these genes might require Opi1p for negative regulation. Our data also demonstrated a role for Opi1p in $IC_{50}$-mediated activation of gene expression. This was shown by the 6.2-fold repression of the $IC_{50}$-activated gene *MSC2* in the *opi1*Δ mutant in the presence of $IC_{50}$ (Table II). Two other $IC_{50}$-activated genes, *MMP1* and *OPT1*, were moderately affected by the loss of Opi1p function, exhibiting at least 2-fold repression in *opi1*Δ,

TABLE VI
*Genes that require Ino4p, but have little or no dependence on Opi1p and Ino2p for regulation in $IC_{50}$ based on the 3-fold cutoff*

| ORF[a] code | Gene name | Fold change: WT + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *opi1*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino2*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino4*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Gene description |
|---|---|---|---|---|---|---|
| Genes that require Ino4p for negative regulation | | | | | | |
| YFL014W | *HSP12* | 1.0 | 1.1 | 2.7 | 3.2 | Heat shock protein |
| YGL156W | *AMS1* | 1.0 | 1.9 | 2.8 | 3.2 | α-Mannosidase |
| YOR120W | *GCY1* | 1.0 | 1.8 | 2.8 | 3.2 | Aldehyde reductase, alcohol dehydrogenase |
| YMR107W | | 1.0 | −2.2 | 2.9 | 3.1 | Unknown |
| YGR088W | *CTT1* | 1.0 | 1.4 | 2.6 | 3.0 | Catalase |
| YOL155C | | 1.0 | −2.1 | 2.3 | 3.0 | Cell wall organization and biogenesis |

[a] ORF, open reading frame; WT, wild-type.

TABLE VII
*Genes that require Ino2p and Ino4p, but have little or no dependence on Opi1p for regulation in the presence of $IC_{50}$ based on the 3-fold cutoff*

| ORF[a] code | Gene name | Fold change: WT + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *opi1*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino2*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Fold change: *ino4*Δ + $IC_{50}$ *vs* WT + $IC_{50}$ | Gene description |
|---|---|---|---|---|---|---|
| Genes that require Ino2p and Ino4p for negative regulation | | | | | | |
| YHR092C | *HXT4* | 1.0 | 1.1 | 6.7 | 3.7 | Fructose, glucose, and mannose transporter |
| YOL053C-A | | 1.0 | 2.1 | 6.0 | 6.4 | Unknown |
| YGL032C | *AGA2* | 1.0 | 2.5 | 4.6 | 4.4 | Cell adhesion receptor |
| YNR044W | *AGA1* | 1.0 | 1.2 | 4.6 | 4.8 | Cell adhesion receptor |
| YCR021C | *HSP30* | 1.0 | 2.8 | 4.2 | 3.2 | Bacterial rhodopsin, heat shock protein |
| YCL027W | *FUS1* | 1.0 | 1.5 | 4.1 | 3.8 | Conjugation with cellular fusion |
| YMR105C | *PGM2* | 1.0 | 1.9 | 4.1 | 3.7 | Phosphoglucomutase |
| YNL160W | *YGP1* | 1.0 | 2.3 | 3.9 | 3.4 | Amino acid metabolism, response to stress |
| YBR072W | *HSP26* | 1.0 | 2.7 | 3.7 | 4.6 | Heat shock protein |
| YHR087W | | 1.0 | 1.7 | 3.6 | 3.7 | Unknown |
| YOR173W | *DCS2* | 1.0 | 1.7 | 3.4 | 3.3 | Unknown |
| YMR250W | *GAD1* | 1.0 | 2.3 | 3.1 | 3.1 | Glutamate decarboxylase |
| YGR248W | *SOL4* | 1.0 | 1.7 | 3.0 | 3.2 | Glucosamine/galactosamine-6-phosphate isomerase |

[a] ORF, open reading frame; WT, wild-type.

suggesting that Opi1p might play a role in the positive regulation of these genes in the presence of $IC_{50}$.

The expression of 33 $IC_{50}$-regulated genes was not affected significantly (exhibiting 2-fold change or less) by the loss of Opi1p function (Table II). These results thus indicate that only a small subset of $IC_{50}$-regulated genes requires Opi1p. Furthermore, for those genes requiring Opi1p, our data demonstrated the role of Opi1p in both negative and positive transcriptional regulation during the $IC_{50}$ response.

*Role of Ino2p and Ino4p in $IC_{50}$-mediated Transcriptional Regulation*—The Ino2p-Ino4p heterodimer was previously shown to be required for derepression of *INO1* with limiting IC concentrations (1, 2). To examine the role of Ino2p and Ino4p in the $IC_{50}$ response, the expression profile of $IC_{50}$-regulated genes was examined in the *ino2*Δ and *ino4*Δ strains and compared with that measured in the wild-type strain under $IC_{50}$ conditions. Of the 50 $IC_{50}$-regulated genes, the expression of only four genes was altered upon the loss of Ino2p or Ino4p. *TPO2* and *AMS1* were highly derepressed, whereas *OPI3* and YDL241W were moderately repressed in both *ino2*Δ and *ino4*Δ strains. These results suggest a role for Ino2p and Ino4p in both positive and negative transcriptional regulation. Interestingly, $IC_{50}$-regulated genes that were dependent on Ino2p and Ino4p exhibited the same expression pattern of down- or up-regulation in both *ino2*Δ and *ino4*Δ strains.

*Quantitative Analysis of $IC_{50}$ Regulation*—To confirm the results of the microarray analyses, a subset of $IC_{50}$-regulated genes was further characterized by semiquantitative (Fig. 1) and real-time (Fig. 2) RT-PCR using specific primers (Table I). The *ACT1* gene, which is not regulated by $IC_{50}$ and is independent of Opi1p, Ino2p, and Ino4p, was used as a control. Analysis of the expression of 12 IC-regulated genes by semiquantitative RT-PCR showed that, in concordance with the microarray data, the transcript levels of *INO1*, *OPI3*, *BIO2*, *BIO3*, *BIO4*, *BIO5*, *VHT1*, *MEP2*, and *SAM2* were reduced in the wild-type strain in response to $IC_{50}$. Conversely, the expression of *GCV1*, *MTD1*, and *OPT1* was induced (Fig. 1). Expression analyses in the *opi1*Δ, *ino2*Δ, and *ino4*Δ strains showed that, in support of our microarray data, the overall transcriptional repression of the *OPI3* gene required functional Opi1p, Ino2p, and Ino4p, with Opi1 acting as a repressor and Ino2p and Ino4p acting as activators. Moreover, RT-PCR analyses confirmed the role of Opi1p as a repressor in the transcriptional repression of *INO1* and *SAM2* and as an activator in the transcriptional activation of *OPT1*, *GCV1*, and *MTD1* in response to $IC_{50}$. Unlike *OPI3*, none of these five $IC_{50}$-regulated genes required Ino2p or Ino4p for their $IC_{50}$-mediated transcriptional regulation. The six other genes analyzed by semiquantitative RT-PCR showed little or no dependence on Opi1p, Ino2p, or Ino4p in response to $IC_{50}$ (Fig. 1). The expression of *INO1*, *BIO5*, and *OPT1* was further analyzed and quantified by real-time PCR in the wild-type and *opi1*Δ, *ino2*Δ, and *ino4*Δ mutant backgrounds. This assay revealed the same pattern of regulation for these genes as that shown by microarray analysis and semiquantitative RT-PCR. With the exception of *INO1*, which was found to be repressed in the wild-type strain by 466.5-fold and derepressed in *opi1*Δ by 489.5-fold using real-time PCR analysis compared with 46.9- and 56.7-fold, respectively, using microarray analyses, most likely due to the difference in sensitivity between the two assays, the repression and derepression ratios for *BIO5* and *OPT1* were almost identical between the two experimental assays (Figs. 2 and 3).

*Opi1p, Ino2p, and Ino4p Are Global Regulators of Gene Expression*—The data described above show the importance of Opi1p, Ino2p, and Ino4p in the regulation of a subset of genes

whose expression changed by at least 3-fold in the presence of $IC_{50}$ in the wild-type strain. To examine the global transcriptional regulation of genes coordinated by Opi1p, Ino2p, and Ino4p, we compared gene expression levels in the wild-type, *opi1*Δ, *ino2*Δ, and *ino4*Δ strains. Because of the inositol auxotrophy of *ino2*Δ and *ino4*Δ, all four strains were compared under $IC_{50}$ conditions. We found 44 genes that exhibited a change in their expression levels by at least 3-fold in at least one of the three mutants. These genes were classified into five groups based on their requirements for Opi1p and/or Ino2p and/or Ino4p (Table III–VII). Overall, 18 genes were regulated by Opi1p (Tables III and IV), 23 by Ino2p (Tables III, V, and VII), and 22 by Ino4p (Tables III, VI, and VII). Of the 18 Opi1p-regulated genes, 17 required Opi1p for negative regulation, whereas one (*MSC2*) required Opi1p for positive regulation (Tables III and IV). Some genes were moderately affected by the loss of Opi1p, exhibiting at least 2-fold activation (*MFA1*, *STF2*, YOL053c-a, *AGA2*, *HSP30*, *YGP1*, *HSP26*, and *GAD1*) (Tables V and VII) or 2-fold repression (YMR107W and YOL155C) (Table VI) in *opi1*Δ, demonstrating once again a role for Opi1p in negative and positive transcriptional regulation. All 23 Ino2p-regulated genes required Ino2p for negative regulation (Tables III, V, and VII). Similarly, all 22 Ino4p-regulated genes required Ino4p for negative regulation (Tables III, VI, and VII). Most genes that were induced in *ino2*Δ were also induced in *ino4*Δ and vice versa (Tables V and VI), suggesting that both Ino2p and Ino4p are required for their negative regulation. Only a small subset of the 44 genes was regulated by $IC_{50}$ (25%) or involved in membrane biogenesis (14%). Hierarchical clustering of the gene expression data in the wild-type, *opi1*Δ, *ino2*Δ, and *ino4*Δ strains further confirmed the presence of different groups of genes with similar regulatory profiles (Fig. 4). Together, these data suggest that Opi1p, Ino2p, and Ino4p are general transcriptional regulators involved in both negative and positive transcriptional regulation of genes, most of which are not regulated by $IC_{50}$.

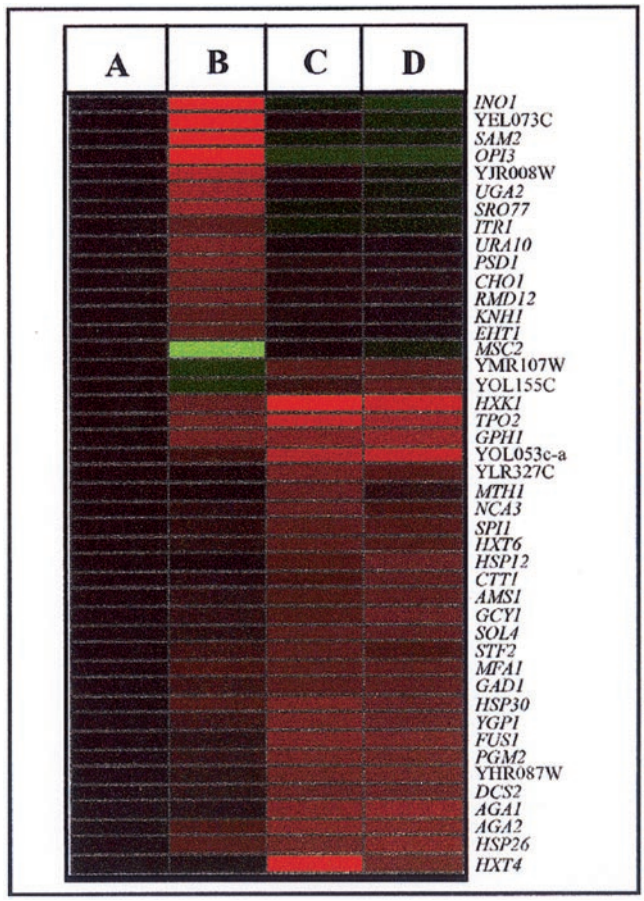


FIG. 4. **Cluster analysis of genes regulated by Opi1p and/or Ino2p and/or Ino4p.** The levels of gene expression in the wild-type (*A*), *opi1*Δ (*B*), *ino2*Δ (*C*), and *ino4*Δ (*D*) strains were compared to the level of expression in the wild-type strain. All strains were grown in $IC_{50}$ as described under "Experimental Procedures." Gene repression is shown in *green*; gene activation is shown in *red*; and unchanged levels are shown in *black*. The -fold ratios and cellular functions of the clustered genes are listed in Tables III–VII.

## DISCUSSION

Yeast strains have evolved to be responsive to changing environmental and nutritional conditions. The transcriptional machineries deployed in response to these changes are different and are triggered by specific sensors able to monitor extracellular or intracellular levels of substrates and affect the expression of genes accordingly (41). Elegant schemes of these regulatory mechanisms have been drawn from thorough biochemical and genetic analyses of glucose (42), amino acid (43–45), and phosphate (46) utilization, to name only a few. Although it is conceivable that similar signaling pathways could be involved in the cellular response to IC, the components of such pathways have not yet been identified. In this study, we have monitored the global response of yeast cells to $IC_{50}$ and further characterized the importance of transcriptional regulators Opi1p, Ino2p, and Ino4p in the regulation of $IC_{50}$-regulated genes. Interestingly, of the 6351 yeast genes analyzed, only 50 were found to be either repressed or induced by at least 3-fold by $IC_{50}$, including *INO1*, *OPI3*, and *PSD1*, previously reported to be repressed by these soluble precursors. Other known IC-repressed genes were repressed by at least 2-fold by $IC_{50}$, such as *ITR1* (2.8-fold), *CHO1* (2.8-fold), *CKI1* (2.5-fold), *INO2* (2.4-fold), *CDS1* (2.2-fold), *ACC1* (2.1-fold), and *INO4* (2.0-fold), whereas other genes were repressed by <2-fold (*FAS1*, *CPT1*, *PGS1*, and *CHO2*) under our conditions. The expression of these genes might require different concentrations of inositol or might be only transiently regulated by IC.

An important finding of our analysis was the discovery of a subset of genes whose expression was significantly activated by $IC_{50}$. Of the 50 $IC_{50}$-regulated genes, 11 were activated by these precursors. Eight of these genes encode proteins that are involved in one-carbon compound metabolism and methionine synthesis, cell wall organization and biogenesis, zinc ion homeostasis, and ethanol fermentation, and the other three genes encode proteins with unknown function. Whereas previous studies have shown that the activity and/or transcription of *DPP1*, *AUR1*, and *INM1* is increased by inositol (26, 29), the expression of these genes was unaffected under our experimental conditions. The differences in inositol and/or choline concentrations between the two studies might account for these differences.

Analysis of gene expression of the $IC_{50}$-regulated genes in *opi1*Δ, *ino2*Δ, and *ino4*Δ showed that only a small subset of these genes required Opi1p, Ino2p, or Ino4p for the $IC_{50}$ response, suggesting that the $IC_{50}$ response involves additional factors, the identity of which is not yet known. Furthermore, among the 39 $IC_{50}$-repressed genes, only 10 contain a copy of the $UAS_{INO}$ sequence in their promoter region, suggesting novel regulatory mechanisms. However, *in silico* analysis failed to identify any specific new or known common motifs in the regulatory regions of those genes. Of the $IC_{50}$-regulated genes, 34% required Opi1p, and 8% required Ino2p and Ino4p. Interestingly, 15 of the 18 Opi1p-regulated genes found in our microarray analysis were also found to be regulated by Opi1p in a previous study, which compared the transcriptional profiles of the wild-type and *opi1*Δ strains grown in rich medium

(47). Our results support previous reports implicating Opi1p in the transcriptional repression of phospholipid-synthesizing genes. However, the role of Opi1p was not limited to negative regulation of genes involved in membrane biogenesis. The cation transporter gene *MSC2* was found to be repressed in *opi1*Δ, suggesting a role for Opi1p in its positive regulation. Overall, our results show that only a small number of genes require Opi1p, Ino2p, or Ino4p for the $IC_{50}$ response and that Opi1p, Ino2p, and Ino4p are involved in both positive and negative regulation of gene expression.

A global view of gene regulation in the wild-type, *opi1*Δ, *ino2*Δ, and *ino4*Δ strains revealed that Opi1p, Ino2p, and Ino4p are global regulators of gene expression, affecting the expression of a large number of genes, only a subset of which is regulated by IC or is involved in phospholipid biosynthesis. Most of the genes regulated by Opi1p, Ino2p, or Ino4p were induced in *opi1*Δ, *ino2*Δ, or *ino4*Δ. Conversely, a small number of Opi1p-, Ino2p-, or Ino4p-dependent genes were moderately repressed in *opi1*Δ, *ino2*Δ, or *ino4*Δ. These results suggest that Opi1p, Ino2p, and Ino4p are general regulators involved in negative and positive transcriptional regulation. Genes that required both Ino2p and Ino4p exhibited the same pattern of down- or up-regulation in both *ino2*Δ and *ino4*Δ strains, suggesting that Ino2p and Ino4p have similar roles and possibly function together as a heterodimeric positive or negative regulatory complex.

In addition to genes involved in phospholipid biogenesis, our study revealed new IC-regulated genes that are involved in various other cellular metabolic pathways. Particularly, all of the known yeast genes involved in biotin biosynthesis (*BIO3*, *BIO4*, and *BIO2*) and the transport of biotin (*VHT1*) and its precursor 7-keto-8-aminopelargonic acid (*BIO5*) were highly repressed by $IC_{50}$. Interestingly, *SAM2*, which catalyzes the formation of *S*-adenosylmethionine (an amino group donor for biotin synthesis) from methionine), *SAM3* (encoding the *S*-adenosylmethionine transporter), *ACC1* (encoding acetyl-CoA carboxylase, which requires biotin as a cofactor), and *BPL1* (encoding a biotin:protein ligase) were also repressed by at least 2-fold in the presence of $IC_{50}$. In conclusion, these studies provide a better understanding of the yeast response to IC; demonstrate the IC-mediated repression and activation of a number of genes involved in different metabolic pathways; suggest the involvement of other regulators during the IC response; and reveal new roles for Opi1p, Ino2p, and Ino4p in the global regulation of gene expression in yeast.

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