The yeast inositol-sensitive upstream activating sequence, UAS\textsubscript{INO}, responds to nitrogen availability

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ABSTRACT

The \textit{INO1} gene of yeast is expressed in logarithmically growing, wild-type cells when inositol is absent from the medium. However, the \textit{INO1} gene is repressed when inositol is present during logarithmic growth and it is also repressed as cells enter stationary phase whether inositol is present or not. In this report, we demonstrate that transient nitrogen limitation also causes \textit{INO1} repression. The repression of \textit{INO1} in response to nitrogen limitation shares many features in common with repression in response to the presence of inositol. Specifically, the response to nitrogen limitation is dependent upon the presence of a functional \textit{OPI1} gene product, it requires ongoing phosphatidylcholine biosynthesis and it is mediated by the repeated element, UAS\textsubscript{INO}, found in the promoter of \textit{INO1} and other co-regulated genes of phospholipid biosynthesis. Thus, we propose that repression of \textit{INO1} in response to inositol and in response to nitrogen limitation occurs via a common mechanism that is sensitive to the status of ongoing phospholipid metabolism.

INTRODUCTION

Structural genes encoding a number of phospholipid biosynthetic enzymes in the yeast \textit{Saccharomyces cerevisiae} show complex transcriptional regulation in response to the availability of the phospholipid precursors, inositol and choline (reviewed in 1–3). The most highly regulated of these genes is \textit{INO1}, the structural gene for inositol-1-phosphate (I-1-P) synthase (4,5). However, a large number of genes encoding other enzymes of phospholipid biosynthesis show similar regulation (reviewed in 1–3,6,7). The basic pattern of regulation is as follows. During the logarithmic phase of growth when inositol is absent from the growth medium, \textit{INO1} and other coordinately regulated genes are derepressed. If inositol is added to the growth medium, these genes are repressed and addition of choline when inositol is present leads to further repression. However, addition of choline by itself has little or no effect.

A repeated element (consensus 5\'–CATGTGAAAT–3\') first detected in the promoter of the \textit{INO1} gene (8,9) known as the inositol-sensitive upstream activated sequence (UAS\textsubscript{INO}) has been detected in the promoters of all such co-regulated genes of \textit{S.cerevisiae} (1–3). This element is the binding site for a heterodimer composed of the products of the \textit{INO2} and \textit{INO4} regulatory genes (9–12). The \textit{INO2} and \textit{INO4} gene products (Ino2p and Ino4p) contain the basic helix–loop–helix (bHLH) DNA binding motif and their binding site, UAS\textsubscript{INO}, contains within it the canonical bHLH site: CANNTG. Strains containing \textit{ino2} and \textit{ino4} mutations are inositol auxotrophs and display other abnormalities of phospholipid metabolism due to failure to derepress the \textit{INO1} gene and other co-regulated genes of lipid metabolism (13). Mutations at a third locus, \textit{OPI1}, lead to constitutive overexpression of \textit{INO1} and a consequent overproduction of inositol (Opi\textsuperscript{+}) phenotype (14). The \textit{OPI1} gene product also exerts its effect through UAS\textsubscript{INO} (11) but it has not yet been established whether the effect of the \textit{OPI1} gene product (Opi1p) on the co-regulated genes is direct or indirect (15). Opi1p contains within it a leucine zipper and polyglutamine stretches (16), both of which are motifs that are commonly found in DNA binding proteins, but Opi1p does not appear to bind directly to DNA (15).

Phospholipid biosynthesis in general decreases and phospholipid biosynthetic enzymes are repressed when yeast cells enter stationary phase (17). Yeast cells exhibit many responses as they enter stationary phase, including arrest in an unbudded state, changes in cell wall structure and accumulation of storage carbohydrates (18). Metabolic changes resembling those observed upon entry into stationary phase are observed when yeast cells growing in the presence of glucose are starved for an essential nutrient such as nitrogen, phosphorus or sulfur (19). These changes have been proposed to be responses to a signal transduction pathway called the fermentable growth medium (FGM)-induced pathway (20). Since the \textit{INO1} gene is repressed as cells approach stationary phase in glucose-containing medium (21–23), even when inositol is absent, we explored the response of \textit{INO1} to transient and total nitrogen limitation in the presence of glucose and other essential nutrients. We report that \textit{INO1} promoter is exquisitely sensitive to nitrogen limitation and that this response is mediated by UAS\textsubscript{INO} and requires the participation of Opi1p. Furthermore, repression of the \textit{INO1} gene in response to nitrogen limitation, similar to its repression in response to the presence of inositol, requires ongoing phosphatidylcholine synthesis.

MATERIALS AND METHODS

Materials

Sources of materials were: [\(\text{\textsuperscript{32}}\text{P}\)]cytidine 5'-triphosphate (sp. act. 800 Ci/mmol), DuPont NEN; nitrocellulose, Schleicher & Schull; SP6/T7 Transcription Kit, Boehringer Mannheim. All other materials were reagent grade or better.

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Strains and plasmids used

The genotypes of the strains of *S. cerevisiae* yeasts used in this study are described in Table 1. Construction and description of plasmid vectors for heterologous reporter gene expression driven by fragments of the INO1 promoter can be found in Lopes et al. (5,24) (pJH334 and pKS102) and Slekar and Henry (25) (pKH200). INO1–lacZ construct pJH334 contains ∼1 kb of INO1 sequence including 132 amino acids from the I-1-P synthase N-terminus and 543 nt 5′ to the start of transcription ligated to lacZ. This construct was linearized and integrated into the ura3 locus of strain W303-1A to create BRS1069 (5). Construct pKS102 is an episomal INO1–CYC1–lacZ plasmid containing nt −259 to −154 of the INO1 5′ region (5). Construct pKH200 contains sequences identical to the first native 10 bp UASINO element (corresponding to the first UASINO on the pKS102 plasmid) placed upstream of the heterologous reporter gene CYC–lacZ′ (25). Yeast transformation was performed by the lithium acetate method (26) with minor modifications.

Table 1. *Saccharomyces cerevisiae* strains used in this study

<table>
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<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source/reference</th>
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<tr>
<td>W303-1A</td>
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<tr>
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<td>(21)</td>
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</table>

Culture conditions

Yeast strains were routinely maintained on YEPD medium (1% yeast extract, 2% Bactopeptone, 3% glucose). Strains containing episomal plasmids were maintained and grown on vitamin defined yeast synthetic medium, YNBv, as described below, with the omission of uracil to maintain selective pressure. Synthetic medium with yeast nitrogen base and vitamins (YNBv) contained 30 g/l glucose, 5 g/l ammonium sulfate, 1 g/l potassium phosphate monobasic, 0.5 g/l magnesium sulfate, 0.1 g/l sodium chloride, 0.1 g/l calcium chloride, trace elements and vitamins as previously described (27) and the following mixture of amino acids and bases: 20 mg/l adenine, 20 mg/l arginine, 20 mg/l histidine, 60 mg/l leucine, 230 mg/l lysine, 20 mg/l methionine, 300 mg/l threonine, 20 mg/l tryptophan and 40 mg/l uracil. The above medium does not contain inositol. Where indicated, media were supplemented with 1 mM choline (C*). Medium without ammonium sulfate (YNBv–NH4) contained all components as above described for YNBv medium but ammonium sulfate was omitted. Medium with ammonium sulfate but without the mixture of amino acid and bases is referred to as YNBv–AA. Medium without ammonium sulfate and without the mixture of amino acids and bases listed above is designated YNBv–NH4–AA.

All cultures were grown aerobically at 30°C with shaking. In a typical experiment, the culture was grown to early exponential phase of growth in YNBv medium, cells were collected by filtration, washed and resuspended in medium lacking one of the nutrients (ammonium sulfate, total nitrogen or phosphate). At indicated time points samples were collected and total RNA was isolated.

RNA analyses

RNA probes for northern blot hybridization were synthesized according to the manufacturer’s recommendations for the SP6/T7 Transcription Kit (Boehringer Mannheim) from plasmids described in Hudak et al. (28), linearized with a restriction enzyme and transcribed with RNA polymerase as follows (plasmid/restriction enzyme/RNA polymerase): pAB309/EcoRI/SP6 (TCM1); pH310/HindIII/T7 (INO1); pYCl101/EcoRI/SP6 (lacZ). RNA was isolated from yeast using glass bead disruption and hot phenol extraction (29). Northern hybridization was performed essentially as described by Hirsch and Henry (4) and described in Materials and Methods. The quantity of INO1 or lacZ RNA was normalized against AMBIS 4000 PhosphorImager (AMBIS Inc.) or densitometry.

RESULTS

Response of the *INO1* gene to nitrogen limitation

Since *INO1* gene expression is repressed in wild-type cells, even in the absence of inositol, as they enter stationary phase (21), we reasoned that starvation for essential nutrients might be responsible for this effect. To explore the response of the *INO1* gene to nitrogen limitation, wild-type strain W303-1A was grown in vitamin defined synthetic yeast medium without inositol containing 5 g/l ammonium sulfate (YNBv). Early in the exponential phase of growth, yeast cultures were transferred by filtration, as described in Materials and Methods, to growth medium lacking ammonium sulfate (YNBv−NH4). Both YNBv and YNBv−NH4 media contain the mixture of amino acids and bases, described in Materials and Methods. The response of the wild-type (W303-1A) yeast culture to the change from a readily used nitrogen source (ammonia) to poorly used nitrogen sources (supplied by the mixture of amino acids and bases) (30) was a slower growth rate (Fig. 1A). However, this shift produced only a minor change in the final density achieved by the culture after prolonged growth and there was no apparent cell cycle arrest during this shift as assessed by ratio of budded versus unbudded cells in the course of the shift (data not shown).

Expression of the *INO1* gene was strongly repressed immediately following the shift to the alternative nitrogen source (Fig. 1B). This response was transient, however, since *INO1* mRNA levels had begun to rise within 4 h and had returned to their original levels ~7 h after the shift. Presumably, this represents the period of time necessary for adjustment of the culture to the use of the alternative nitrogen sources. During the period of time following the shift to YNBv−NH4 medium while *INO1* expression was repressed, there was relatively little effect on the transcript of the *TCM1* (ribosomal protein gene) used as a control for RNA loading (Fig. 1B). Since the product of the *OPI1* gene (Opi1p) is required for repression of *INO1* gene expression in response to inositol (11,16), we tested the level of *INO1* transcript in strain BRS1021, which contains an *opi1* deletion mutation (see Table 1 for complete strain descriptions). In this strain, unlike the wild-type, no repression of the *INO1* gene was observed in response to nitrogen limitation when the culture was transferred from YNBv to YNBv−NH4 medium (Fig. 2).

*INO1* expression was also studied during nitrogen starvation when no alternative nitrogen source was available. A wild-type strain requiring no amino acids (WT-lacZ) and a congenic strain...
Figure 1. (A) Growth of the wild-type strain (W303-1A) after transfer to medium containing a poorly used nitrogen source. Cultures were grown in YNBv medium to logarithmic phase \((t = 0)\). At \(t = 0\), the culture was collected by filtration and washed with the medium to which it was to be transferred. One portion of the culture was transferred to YNBv–NH\(_4\) lacking ammonium sulfate. The other portion was transferred to fresh YNBv medium \((l)\). Growth was monitored by optical density. (B) Northern blot analysis of \(INO1\) expression after transfer to medium containing a poorly used nitrogen source. At indicated time points after the transfer to YNBv–NH\(_4\) medium, total RNA was isolated from collected samples and analyzed as described in Materials and Methods. Hybridization with the \(TCM1\) ribosomal protein gene probe serves as an RNA loading control. Quantitation of \(INO1\) expression obtained by densitometry is shown below the blot. The proportion of \(INO1\) expression relative to the \(TCM1\) loading control was set at 100 at time 0. The numbers corresponding to subsequent times represent the percentage of \(INO1\) expression remaining relative to time 0.

containing the \(opi1\) deletion mutation (OP-lacZ) \((21)\) were grown to the early exponential phase of growth and transferred from synthetic medium (without inositol) containing ammonium sulfate \((5 \text{ g/l})\) but no amino acids \((\text{YNBv–AA})\) to medium without ammonium sulfate and containing no other alternative source of nitrogen \((\text{YNBv–NH}_4\text{–AA})\). Both the wild-type strain and the \(opi1\) mutant underwent growth arrest very shortly after the shift to medium without any nitrogen source (data not shown). \(INO1\) gene expression was immediately and strongly repressed in the wild-type strain (Fig. 3) but remained derepressed in the congenic \(opi1\) derivative (Fig. 4). The \(TCM1\) gene was also repressed in both strains immediately after the transfer of culture to the medium without any nitrogen source. The repression of \(TCM1\) expression under conditions of total nitrogen withdrawal (Figs 3 and 4) is in sharp contrast to the continuing expression of \(TCM1\) during transient nitrogen starvation in both the \(opi1\) and wild-type strains (Figs 1 and 2). It is noteworthy, however, that \(INO1\) expression continued even after growth arrest in the \(opi1\) strain starved for nitrogen (Fig. 4). This is consistent with the report by Jiranek et al. \((21)\) that \(INO1\) expression continues in \(opi1\) mutants well into stationary phase.

Ongoing phosphatidylcholine synthesis is essential for \(INO1\) repression due to nitrogen limitation

cho2 mutants, which are defective in the first methylation step en route to phosphatidylcholine (PC) (the pathway for phospholipid biosynthesis is shown in Fig. 5), and other mutants with defects in the methylation pathway leading to PC, display altered regulation of \(INO1\) in response to the soluble precursors of phospholipid biosynthesis, inositol and choline \((22,31,32)\). These
mutants have an inositol excretion phenotype (Opi−) (14) that is eliminated when choline is supplied in the growth medium (22,32). The cho2 mutants exhibit derepressed INO1 levels even in the presence of inositol unless they are supplied with exogenous choline (22). Repression of the INO1 gene in response to inositol in cho2 mutants is restored by supplementation with the precursors monomethylethanolamine (MME) and dimethyl-ethanolamine (DME) (32) which can be incorporated via the CDP choline pathway (33) into phospholipid. Since the cho2 mutation blocks the conversion of phosphatidylethanolamine to phosphatidyl-dimethylethanolamine, all three precursors enter phospholipid biosynthesis downstream of the cho2 genetic lesion and restore PC biosynthesis (Fig. 5).

In the present study, the cho2 strain was grown in defined yeast synthetic medium lacking inositol but containing ammonium sulfate (YNBv), with (C+) or without (C−) 1 mM choline supplement as indicated. Early in the exponential phase of growth, cultures were transferred to the respective media without ammonium sulfate but containing the mixture of amino acids and bases (YNBv–NH4). In the presence of choline, INO1 expression in the cho2 mutant follows the same general pattern of transient repression of INO1 expression in the absence of ammonium sulfate in the medium (YNBv–NH4) (Fig. 6) that is observed in the wild-type strain (Fig. 1B). The repression is not quite as complete as is observed in the wild-type strain. However, in the cho2 strain, in the absence of choline (Fig. 6), the INO1 gene exhibits much less repression than is characteristic of the wild-type response to nitrogen limitation or is observed in the cho2 strain itself when choline is present.

**Effect of elements within the promoter of the INO1 gene**

Previously, a construct containing 543 nt of the INO1 promoter upstream of the translational start site was shown to be sufficient to drive regulated expression in wild-type yeast cells of a fusion construct containing the *E. coli* lacZ gene. This construct is regulated like the native INO1 gene in response to the availability of the precursors inositol and choline (5). We asked whether this construct also showed repression comparable to the
Expression of INO1-lacZ constructs fused to smaller fragments of the INO1 promoter in response to nitrogen limitation.

**Figure 6.** Northern blot analysis of INO1 expression in a cho2 strain, following transfer to medium with a poorly used nitrogen source, in the presence and absence of choline. The cho2 strain was grown in YNBv to mid-logarithmic phase with and without choline (C⁺ and C⁻, respectively). The cultures were then transferred by filtration from complete synthetic medium (YNBv) to medium without ammonium sulfate (YNBv–NH₄). Choline supplementation (C⁺) or lack thereof (C⁻) was maintained at transfer. At indicated time points after the transfer, total RNA was isolated and subjected to northern blot analysis. Hybridization with the TCM1 ribosomal protein gene probe serves as an RNA loading control. Quantitation of INO1 expression obtained by densitometry is shown below the blot. The proportion of INO1 expression relative to the TCM1 loading control was set at 100 at time 0. The numbers corresponding to subsequent times represent the percentage of INO1 expression remaining relative to time 0.

To further characterize the cis-acting elements within the INO1 promoter responsible for the regulation of the INO1 gene in response to nitrogen limitation, we analyzed expression of fusion constructs containing smaller fragments of the INO1 promoter. In these studies, lacZ expression was driven by fragments of the INO1 promoter fused to lacZ contained on an episomal plasmid. Expression of lacZ measured by northern blot was compared to the native INO1 transcript (Fig. 7). However, the absolute levels of lacZ expression were approximately three times higher from the pKH200 construct under both repressed and derepressed conditions as compared to lacZ mRNA levels derived from the INO1 promoter (11,24,25), as well as a copy of an upstream repression sequence (URS1) (24), which has been reported in the promoters of many yeast genes (34). Construct pKH200 contains sequence identical to the 10 bp UASINO element found in position –244 to –235 of the INO1 promoter (the first of two UASINO elements present in plasmid pKS102) (25). The pattern of transcription of the lacZ fusion construct driven by the fragments of the INO1 promoter containing the two UASINO elements together with URS1 (pKS102) was similar to the response of the native INO1 gene under the same conditions (Fig. 8). Similarly, lacZ transcription driven only by the first of these UASINO elements (pKH200) also mirrors the pattern of INO1 expression of the native INO1 transcript (Fig. 9). However, the absolute levels of lacZ expression were approximately three times higher from the pKH200 construct under both repressed and derepressed conditions as compared to lacZ mRNA levels derived from pKS102. The effect of nitrogen limitation on expression of these INO1 constructs is similar to the effects obtained when these same constructs were studied in conjunction with repression in response to inositol (24).

**DISCUSSION**

Nutrient starvation is a fundamental condition that signals microorganisms to slow cellular metabolism, cease cell division and enter stationary phase. In glucose grown cells, starvation for nitrogen triggers the fermentable growth medium (FGM) signal transduction pathway which results in the appearance of stationary
Nutrients such as nitrogen and phosphate. The must be regulated at some level by the availability of basic production of membrane constituents, including phospholipids, growth and metabolism with membrane biogenesis requires that of the stationary characteristics (19). The coordination of cell Re-feeding with the limiting nutrient results in rapid disappearance ribosomal protein genes and induction of heat shock genes.

...arrest including: glycogen and trehalose accumulation, repression of the cell cycle, enter G0 and exhibit characteristics of stationary phase. Nutrient-starved cells arrest in the G1 phase of the... the adjustment of cellular metabolism to the use of alternative nitrogen sources (30), INO1 transcription was repressed transiently while the level of the TCM1 transcript was only slightly affected (Fig 1). Thus, the response of the INO1 gene to transient nitrogen limitation was more sensitive than the response of the TCM1 gene. Furthermore, when the opi1 gene is deleted, INO1 expression continues even when nitrogen is completely absent from the medium (Fig. 4), but the opi1 mutation has no effect on TCM1 expression (compare Figs 3 and 4) under these conditions. These observations suggest that INO1 repression in response to nutrient limitation is separate from the regulatory mechanism controlling the TCM1 gene under these same conditions.

By studying lacZ fusion constructs whose expression was driven by portions of the INO1 promoter, we have shown that the elements of the INO1 promoter necessary for repression in response to inositol (UASINO) are also sufficient to drive repression of INO1 in response to transient nitrogen limitation (Figs 7–9). Thus, it appears that INO1 sensitivity to nitrogen limitation is controlled by UASINO, the same element that controls repression in response to inositol (5,9,35). The INO1 promoter contains two active UASINO elements (5,9,35) and an active URS1 element (24). The INO1 promoter has been extensively studied with respect to each of the UASINO elements and the URS1 element. In a previous study, binding of a putative URS binding factor with the URS1 element in the INO1 promoter was analyzed and the activity was shown to be competed by the CARI URS1 sequence (24). The URS1 element has been shown to affect the overall level of repression of INO1, but UASINO is solely responsible for mediating the response to inositol (11,24).

The presence of a single synthetic UASINO element in a completely heterologous reporter gene construct is completely sufficient to confer repression in response to inositol and to place...
the construct under OPI1 control (11). In previous studies, we have shown that the OPI1 gene product is required not only for repression of INO1 in response to inositol (11) but also for its repression as cells enter stationary phase (21). Expression of INO1 is also very sensitive to mutations affecting transcription globally (reviewed in 2). For example, the SIN3 gene product, which is a component of a large complex involved in histone deacetylation, also affects INO1 expression (28). The effect of sin3 mutations on INO1 expression was shown to be mediated both by URS1 and UASINO (25). Most recently, INO1 expression has also been shown to be influenced by two major signal transduction pathways, the unfolded protein response pathway (36) and the glucose response pathway (37,38). In both cases, mutations in protein kinases, IRE1 and SNF1/SNF4, respectively, lead to loss of INO1 expression and resulted in an Ino− phenotype. In both cases, deletion of the OPI1 gene restored INO1 expression but not its regulation in response to inositol (36,37). Thus, the regulation of INO1 is very complex and is influenced by the overall status of the cellular transcription apparatus and several major signal transducing pathways. In this report, we have shown that the INO1 gene is rapidly repressed in response to nitrogen limitation and that this response appears to be controlled by the same mechanism that controls repression in response to inositol, namely the response is conferred by UASINO and requires a functioning OPI1 gene product. The fact that deprivation of nitrogen triggers repression of INO1 via the control of UASINO provides information relevant to potential mechanisms for the observed repression of UASINO-containing genes upon entry into stationary phase (21,23).

Griac et al. (22) showed that the kinetics of repression of UASINO-containing genes upon entry into stationary phase was altered in cells containing mutations affecting PC biosynthesis. Specifically, it was shown that cho2 mutants, which have a defect in phospholipid methylation (Fig. 5), exhibited slower kinetics of repression upon entry into stationary phase when starved for choline (22). Yeast strains carrying the cho2 mutation also show aberrant transcriptional regulation in response to inositol unless a metabolite downstream of the genetic block in PC biosynthesis is supplied exogenously (22,32). Specifically, cho2 cells fail to repress the INO1 gene and other co-regulated genes of phospholipid biosynthesis in response to inositol, unless choline (or MME or DME) is also supplied. In this study, we observed that when cho2 cells were grown in the absence of choline, as well as inositol, and were subsequently transferred to medium lacking ammonium sulfate, as well as choline and inositol, the INO1 gene did not exhibit transient repression in response to nitrogen limitation. However, when the same experiment was repeated with cho2 cells grown in medium containing choline but lacking inositol, the INO1 gene was repressed, following transfer to nitrogen limiting medium. Thus, growth of cho2 cells in the absence of choline, a condition that results in reduced PC biosynthesis and an inability to repress the INO1 gene in response to inositol, also results in inability of the cells to repress the INO1 gene in response to nitrogen limitation. Restoration of PC biosynthesis by supplying exogenous choline restores repression in response to both signals, i.e. inositol (22) and nitrogen limitation (Fig. 6). This observation suggests that the metabolic signals for the two types of response (i.e. by INO1 repression in response to inositol or to nitrogen limitation) may be identical. This connection between inositol and nitrogen metabolism is currently being explored.

We have recently proposed a model for the transcriptional regulation of the phospholipid biosynthetic genes which can account for repression in response to inositol/choline availability in logarithmically growing cells (2,40). Our model proposes that the build-up of a precursor early in phospholipid biosynthesis, either phosphatidic acid (PA) or a precursor closely related to PA (Fig. 5), produces a signal that results in derepression of the co-regulated genes containing UASINO. Cellular levels of PA are influenced by at least three ongoing metabolic processes: (i) the rate of de novo synthesis of PA from glycerol 3-phosphate and fatty acids (Fig. 5); (ii) the rate of production of PA by phospholipase D-mediated phospholipid turnover (40,41); (iii) the rate of utilization of PA in downstream reactions of phospholipid biosynthesis (42). Inositol, when available in the growth medium, draws on the pool of cytidine diphosphate diacylglycerol (CDP-DG) which, in turn, draws upon PA. Choline draws upon the availability of diacylglycerol which is, in turn, derived from PA (Fig. 5). Our model proposes that when the rate of PA production, via de novo synthesis and/or turnover of existing lipids, exceeds its utilization in downstream reactions, INO1 and co-regulated UASINO-containing genes will be derepressed (2). The cho2 mutation blocks the major pathway leading from PA through CDP-DG and phosphatidylserine to PC (Fig. 5). Consistent with the model, in cho2 cells growing in the absence of choline, neither inositol (22) nor transient nitrogen limitation (Fig. 6) causes repression of INO1. However, when choline is supplied to cho2 mutants, it permits the synthesis of PC drawing on diacylglycerol downstream of PA (Fig. 5) and simultaneously restores repression of INO1, both in response to inositol (22) and nitrogen deprivation (Fig. 6). The results depicted in Figure 6 clearly show that the metabolic signal that triggers INO1 repression in response to transient nitrogen limitation, like the response to inositol availability, is influenced by the pattern of phospholipid metabolism.

The experiments reported here show that INO1 is rapidly repressed in response to nitrogen limitation. Moreover, this regulation shares common features with the regulation that occurs in response to inositol and choline (8). (i) Repression in response to both types of metabolic signals (i.e. inositol and nitrogen limitation) are dependent on a functional OPI1 gene product; (ii) ongoing PC biosynthesis is necessary for proper wild-type regulation in both cases; (iii) in both cases, the minimal promoter requirement for proper regulation of a heterologous system is the 10 bp UASINO consensus sequence. These common features suggest that a single regulatory mechanism is involved in controlling repression of the INO1 gene to nitrogen limitation and inositol/choline availability. This hypothesis and the interconnection between inositol and nitrogen metabolism are currently being explored in our laboratory.

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