Multiple Regulatory Roles of a Novel \textit{Saccharomyces cerevisiae} Protein, Encoded by \textit{YOL002c}, in Lipid and Phosphate Metabolism*

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The yeast \textit{Saccharomyces cerevisiae} is able to survive and grow on a wide range of media due to its ability to activate pathways that enable utilization of alternate carbon sources. This organism responds to a diverse array of signals, and many of the responses involve the regulation of gene expression. This transcriptional regulation results in a corresponding adjustment in signaling systems that are activated by the switch in carbon source supplied for growth. Thus, the ability of yeast to utilize various carbon sources is highly regulated, and the expression of genes required for the utilization of specific carbon sources has been a major topic of study for many years (1). Much is known regarding the regulatory mechanisms that occur when yeast is grown on glucose. Many proteins are dispensable under these conditions; therefore, the expression of genes encoding such proteins is transcriptionally repressed. These include genes required for the metabolism of carbon sources that are utilized less efficiently than glucose, such as glycerol (\textit{GUT} (2)), galactose (\textit{GAL} (3)), ethanol (\textit{ADH2} (4)), and fatty acids (\textit{POX1} (5)). The expression of genes encoding enzymes involved in the utilization of carbon sources other than glucose are often up-regulated in the presence of the appropriate nutrient. The shift from a glucose- to an oleate-containing medium leads to induction of a number of enzyme activities, including the peroxisomal enzymes involved in the \(\beta\)-oxidation pathway, and it also leads to peroxisome proliferation (6). A sequence motif within the promoter region of genes encoding these proteins was demonstrated to be the binding site for protein(s), as then unidentified, responsible for oleate induction, and was termed the glate-response element (ORE)\(^1\) (7).

In addition to fatty acid-dependent transcriptional activation of specific genes, other genes are transcriptionally repressed in response to a change in carbon source. The \textit{OLE1} gene encodes \(\Delta-9\) fatty acid desaturase, an enzyme involved in the formation of unsaturated fatty acids. \textit{OLE1} expression is repressed when certain unsaturated fatty acids such as oleate are added to the growth medium, but it is induced when cells are grown in the presence of a saturated fatty acid (8, 9). Recently, two genes, \textit{MGA2} and \textit{SPT23}, were implicated in the transcription of several genes in \textit{S. cerevisiae}, including \textit{OLE1}. The loss of function of both \textit{MGA2} and \textit{SPT23} results in a 15-fold decrease in the level of the \textit{OLE1} transcript (10). The active Spt23p transcription factor is synthesized in the form of an inactive membrane-bound precursor, and the active form is generated by a processing step that is regulated by unsaturated fatty acids (11).

\textit{POXI} encodes fatty acyl-CoA oxidase, the rate-limiting enzyme of the peroxisomal \(\beta\)-oxidation cycle. Previously, we extensively mapped the promoter region of \textit{POXI} and have demonstrated that there are at least three regulatory elements in this promoter (12, 13), one of which is an ORE. We and others (14–16) subsequently identified two transcription factors, Oaf1p and Pip2p, that bind to the ORE and mediate oleate-dependent transcriptional activation. The completion of the yeast genome sequencing project in 1996 provided us with the possibility of finding additional genes that contain an ORE in their promoters. We demonstrated that more than 20 genes, encoding proteins with various subcellular locations, are regulated by the Oaf1p/Pip2p transcription factors (17). In addition to genes encoding known peroxisomal, mitochondrial, and nuclear proteins, we found that several open reading frames (ORFs), encoding proteins of unknown location and function, are also regulated by Oaf1p and Pip2p.

The gene \textit{YOL002c} was among the ORFs that we found to contain an ORE (17). This gene encodes a putative protein that is predicted to contain seven transmembrane domains. In this current study we demonstrate that the expression of \textit{YOL002c} is highly induced in cells grown in the presence of a medium chain length saturated fatty acid, such as myristate. We further show that a strain in which the \textit{YOL002c} gene is disrupted grows poorly in medium containing myristate as the main carbon source, and that \textit{yol002c}\(\Delta\) cells are resistant to the

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\(^1\) The abbreviations used are: ORE, oleate-response element; ORF, open reading frame.
polysaccharide antibiotic, nystatin. In addition, we found that high chain length inorganic polyphosphate accumulates to higher levels in the disruption strain when compared with the wild-type strain. Finally, we show that 32PPO4 is taken up by yolk002Δ cells at a higher rate than in wild-type cells and that acid phosphatase activity is constitutively expressed in yolk002Δ cells, even in phosphate-rich medium. Phosphate is an essential nutrient for all organisms including yeast; thus a tight regulatory mechanism for acquisition, storage, and release of phosphate has evolved. When phosphate becomes limiting in the growth media, there is an increased production of a high affinity phosphate transporter and of secreted phosphatase(s) that scavenge phosphate from the environment. The signal transduction pathway involved in the regulation of phosphate-responsive genes is complex and involves over 20 genes (for review see Ref. 18). We suggest that the YOL002c protein plays a role in this elaborate pathway.

Taken together, our results suggest that cells lacking YOL002cp display multiple defects in lipid homeostasis as well as in the phosphate levels of the cell.

MATERIALS AND METHODS

Yeast Strains and Media

The yeast strains used in this study are described in Table I. Yeast strains were grown in either YPD (1% yeast extract, 2% peptone, 2% glucose); SD (0.67% yeast nitrogen base without amino acids, 2% glucose); or YPG (1% yeast extract, 2% peptone, 3% glycerol). Liquid-rich media containing fatty acids (YPGFA) were composed of YPG supplemented with 0.1% (v/v) of the respective fatty acid and with 0.25% (v/v) Tween 40. In the case of very long chain fatty acids (C20 and longer), the Tween 40 was substituted with 0.25% Tergitol. YNCF plates contained 0.67% yeast nitrogen base without amino acids, 0.3% casamino acids, 0.25% (v/v) Tween 40% or 0.25% (v/v) Tergitol, and 0.1% (w/v) of the respective fatty acid. In order to prepare low phosphate media (YPD–P), the method of Kaneko et al. (19) was used with minor modifications. Briefly, 20 g of peptone and 10 g of yeast extract were first dissolved in 800 ml of H2O, and 10 ml of 1 M MgSO4 and 10 ml of 30% ammonium hydroxide were then added. The resultant solution was incubated for 30 min at room temperature to form a precipitate and was then filtered through Whatman No. 1 paper. The pH of the filtered solution was adjusted to pH 5.8 using 1 N HCl, and the volume was brought to 900 ml with H2O. The medium was sterilized by autoclaving, and 20% dextrose was added to a final concentration of 2%. To determine Pi, 1 M KH2PO4 was added to a final concentration of 10 mM.

Yeast strains used in this study

| Strain | Genotype | Ref. |
|--------|----------|-----|
| W3031A | MATa leu2 ura3 trp1 ade2 his3 | 38 |
| ΔO1ΔP2 | MATa leu2 pip2::LEU2 ura3 trp1 ade2 his3 oaf1Δ::HIS3 | 17 |
| yolk002Δ | MATa leu2 ura3 trp1 ade2 his3 yolk002::HIS3 | This study |
| ydr492wΔ | MATa leu2 ura3 ydr492w::hisG trp1 ade2 his3 | This study |
| yolk101Δ | MATa leu2 ura3 yolk101c::hisG trp1 ade2 his3 | This study |
| yolk002Δydr492wΔyolk101Δ | MATa leu2 ura3 ydr492w::hisG trp1 ade2 his3 yolk002::HIS3 | This study |
| W002c-BG1 | MATa leu2 ura3 (Ylp357-URA3-YOL002c(ATTG1)-lacZI) trp1 ade2 his3 | This study |
| W006c-BG2 | MATa leu2 ura3 (Ylp357-TRA3-YOL002c(ATTG2)-lacZI) trp1 ade2 his3 | This study |
| ΔO1ΔP2-BG2 | MATa leu2 pip2::LEU2 ura3 (Ylp357-URA3-YOL002c(ATTG2)-lacZI) trp1 ade2 his3 oaf1Δ::HIS3 | This study |
| yolk002Δ-CGI-45 | MATa leu2 ura3 (pRS306-URA3-YOL002c(ATTG2)-CGI-45) trp1 ade2 his3 | This study |
| yolk002::HIS3 | | |
| SCY325 | MATa leu2 ura3 trp1 ade2 his3 | 38 |
| SLΔ | MATa leu2 ura3 trp1 ade2 his3 yolk002::HIS3 | This study |

Plasmids

All recombinant plasmids were created using a combination of PCR and subcloning techniques. The oligonucleotide primers are shown in Table II. Ylp357-002c-ATG1—In order to confirm the data obtained by Northern blot analysis for YOL002c-expression, two constructs containing the lacZ sequence under the control of the YOL002c promoter were created. A pair of primers, G9 with G10, was used in a Pfu Turbo-driven PCR to amplify a fragment that contains the 1040-bp promoter region and the predicted initiation codon of YOL002c, immediately followed by a HindIII site. Following amplification, the DNA fragment was cleaved with BamHI and HindIII and then subcloned into the corresponding sites of the vector Ylp357 (21), producing Ylp357-002c-ATG1. This construct failed to produce β-galactosidase activity when introduced into our wild-type strain W3031A (W002c-BG1).

Ylp357-002c-ATG2—To create a fragment that contains the YOL002c promoter and the sequence 30 bp downstream from the predicted initiating ATG, which included a second ATG codon, also immediately followed by a HindIII site, a pair of primers, G9 with G11, was used. After amplification, the DNA fragment was sub克隆 into the
ATG2 DNA was first digested with BamHI and CGI-45 (Table I). CGI-45 HI and fragment containing the promoter region and the second ATG codon of were collected, washed with water, and resuspended in 1 ml of cold sucrose, 0.05% bromphenol blue in 1 M EDTA. Following centrifugation, the pellet was resuspended in 50 μl of H2O. The concentration of total RNA was calculated by measuring the absorbance at 260 nm.

To create a construct that expresses the human gene CGI-45 under the control of the YOL002c promoter, Yip535-002c-ATG2 DNA was digested with BamHI and HindIII, and the 1.1-kb fragment containing the promoter region and HindIII and the 1.1-kb fragment fragment containing the promoter region and the second ATG codon of were collected, washed with water, and resuspended in 1 ml of cold sucrose, 0.05% bromphenol blue in 1 M EDTA. Following centrifugation, the pellet was resuspended in 50 μl of H2O. The concentration of total RNA was calculated by measuring the absorbance at 260 nm.

To compare the gene expression patterns between SCY325 cells grown on YPGM medium, and poly(A)+ RNA was purified according to a hot phenol extraction procedure as described (17). A phenol/chloroform extraction procedure was used to isolate total yeast RNA using Oligotex detergent strips (Qiagen, Valencia, CA), and labeled with a Prime-It RmT kit (Stratagene, La Jolla, CA). Yeast mRNAs were resolved, transferred to nylon membrane, and hybridized as described previously (14). Yeast gene-specific probes were generated by PCR amplification with primers based on sequence from the yeast genome data base (Table II) and total yeast DNA. The PCR products were resolved on a standard 1% agarose gel, purified using a GeneClean kit (Bio 101, Vista, CA), and labeled with a Prime-It RmT kit (Stratagene, La Jolla, CA) and [32P]dCTP. Hybridization and subsequent analyses were performed exactly as described previously (17).

**DNA Microarray Analysis**

To compare the gene expression patterns between SCY325 cells (Table I) and isogenic yeast strains (s2) cells, the Affymetrix Yeast Genome S98 Arrays (YG-98) were used. Both yeast strains were grown on YPGM medium, and poly(A)+ mRNA fractions were then prepared from total yeast RNA as described above. Double-stranded cDNA preparation, synthesis of biotin-labeled cRNA target, hybridization, washing and staining, subsequent scanning of the hybridized array, and data processing were performed as specified in the Affymetrix Gene Chip Expression Analysis Technical Manual.

**Polyphosphate Detection by Gel Electrophoresis**

Pre-cultures were grown at 30 °C in YPD − Pi, media overnight as described (19). The cells were collected, washed with water, and diluted 1:100 in YPD − Pi. After incubation for 6 h at 30 °C, KH2PO4 was added to a final concentration of 10 mM. Following a 2-h incubation, the cells were collected, washed with water, and resuspended in 1 ml of cold LETS buffer (0.01 M Tris-HCl, pH 7.4, 0.01 M EDTA, 0.1 mM L-Cit, 2% SDS). Glass beads (at approximately equal volume) and 700 μl of phenol/chloroform (saturated to pH 5.0) were added to the cells. The cells were vortexed 6 times for 10 s and were then centrifuged at 10,000 rpm for 20 min. The aqueous phase was collected and extracted twice with phenol/chloroform. The RNA, together with the polyphosphates, was precipitated in the presence of 0.2 M LiCl and 2.5 volumes of ethanol. Following centrifugation, the pellet was resuspended in 50 μl of H2O. The concentration of total RNA was calculated by measuring the absorbance at 260 nm.

**P<sub>i</sub> Uptake Experiments**

In order to measure the uptake of Pi cells were grown overnight in YPD − Pi, or YPD + Pi, media. The cells were collected, washed with water, and resuspended in low phosphate synthetic complete medium (SC − Pi), or high phosphate synthetic complete medium (SC + Pi), to a final concentration of A<sub>600</sub> = 0.1. Cells were shaken for 2 h at 30 °C, and then 1 μCi/ml [32P]Pi in KH2PO4 was added to give a final P<sub>i</sub> concentration of 0.1 mM in the assay reaction. Samples were withdrawn at different time points and were filtered through a nitrocellulose filter (Millipore HA, 0.45 μm). The filters were washed with 10 ml of SC − P<sub>i</sub>, medium and were then dried, and the radioactivity collected on each filter was quantitated using a liquid scintillation counter (Beckman LS1801).

**Acid Phosphatase Activity Assay**

In order to measure the acid phosphatase activity, cells were grown overnight at 30 °C in YPD − Pi, or YPD + Pi, media. The cells were collected by centrifugation, washed with water, and resuspended in lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.5 mM CaCl<sub>2</sub>, 0.2% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride). Glass beads (at approximately equal volume) were added, and the tubes were vortexed for 30 min at 4 °C. The tubes were then centrifuged at 10,000 rpm for 20 min, and the supernatant was recovered for phosphatase activity and protein assays. Acid phosphatase was measured using p-nitrophenyl phosphate (Sigma) as a substrate, and the assay was performed using a citrate buffer (90 mM sodium citrate, 10 mM NaCl, pH 4.5). The absorbance at 405 nm was then measured, and the activity was calculated as described (19). The activity was expressed as micromoles of p-nitrophenol liberated by 1 mg of protein in 1 ml of reaction mix.

**RESULTS**

**Expression of YOL002c** — We demonstrated previously that YOL002c is expressed at high levels in glucose-grown cells, whereas the expression is low in cells grown in the presence of oleate (17). Expression in each of these media is reduced in a strain in which the Oaf1p/Pip2p transcription factors are deleted (17). Because the majority of genes that are regulated under the control of Oaf1p and Pip2p are induced in cells grown in the presence of oleate, this result was unexpected. Therefore, we proceeded to examine the expression of YOL002c in cells grown in additional unsaturated or saturated fatty acids. The

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*2 S. Sturley and L. Wilcox, personal communication.*
levels of \textit{YOL002c} were determined by Northern analysis of poly(A)\(^{+}\) RNA isolated from wild-type and \textit{oaf1}Δ\textit{pip2}\(\Delta\) cells grown in glucose, glycerol, or glycerol plus the following fatty acids: C18:1 (oleate), C18:2, C6:0, C14:0, C16:0, C18:0, and C20:0. Our results demonstrated that \textit{YOL002c} is highly induced when cells are grown in the presence of C14:0 (myristate) and that this level of expression is dependent on the Oaf1p/Pip2p transcription factors (Fig. 1).

In order to confirm the expression of \textit{YOL002c} in the presence of different fatty acids, we studied the transcription using a \textit{YOL002c-lacZ} reporter construct. The first construct that we prepared, based on the ORF provided by the yeast genome database, failed to produce any \(\beta\)-galactosidase activity. However, when we prepared an alternative construct in which we fused the second ATG, which is 30 nucleotides downstream from the predicted initiating ATG, with the \textit{lacZ} gene (Fig. 2a), we measured a high level of \(\beta\)-galactosidase activity in extracts from cells grown in the presence of myristate (Fig. 2b). Thus, the correct initiating ATG is downstream from that given in the yeast genome database, and the \textit{YOL002c} protein is 10 amino acids shorter than predicted. The protein contains 317 amino acids and has a predicted molecular mass of 36.3 kDa.

The \(\beta\)-galactosidase activity in extracts from wild-type cells was ~5-fold higher from cells grown in the presence of myristate when compared with glycerol-grown cells (Fig. 2b). Furthermore, we demonstrated that both of these genes are transcriptionally induced by saturated fatty acids. \textit{YDR492w} shows highest expression in cells grown in the presence of C18:0, whereas \textit{YOL101c} is highly expressed in both C18:0 and C20:0. Expression of both of these genes is reduced in the \textit{oaf1}Δ\textit{pip2}\(\Delta\) strain (Fig. 3B).

Through an extended search for proteins homologous to \textit{YOL002c}, we found genes in \textit{Caenorhabditis elegans}, \textit{Drosophila}, and humans (COI-45), each of which encode proteins that show a striking homology to this ORF (for example, \textit{YOL002p} and the human protein CGI-45 share 29% identity) (Fig. 4). Thus far, the function of each of these proteins remains unknown. However, the fact that the proteins are conserved from yeast to man suggests that the encoded proteins have an important role.

\textbf{Phenotype of a \textit{YOL002c} Disruption Strain}—In an attempt to gain insight into the function of the \textit{YOL002c} protein, we prepared a strain in which the gene encoding this protein was disrupted (see “Materials and Methods”). \textit{YOL002p}\(\Delta\) cells appeared to grow at normal rates in YPD medium; however, they grow poorly on plates provided with myristate as the sole carbon source (data not shown). The disruption strain also grows poorly on non-fermentable carbon sources, such as glycerol or lactate (data not shown).

We further found that the \textit{YOL002c} deletion strain exhibits resistance to the polyene antibiotic, nystatin (data not shown). Nystatin forms a complex with sterols in the cell membrane of sensitive organisms, resulting in leakage of essential cellular metabolites (23). Because wild-type yeast strains are sensitive to this antibiotic (24), we hypothesize that deletion of the
YOL002c gene results in a qualitative change in the sterol composition of the cell membrane. Introduction of the human CGI-45 gene into our yol002c/H9004 strain failed to rescue either of these mutant phenotypes (data not shown). Ydr492w/H9004 and yol101c/H9004 cells grew at a similar rate as wild-type cells in the presence of myristate, whereas the growth of these mutant strains in the presence of nystatin was variable.

Since the sequence of the entire S. cerevisiae genome has been available, there have been several genome-wide expression analyses carried out to explore the global response of gene...
expression to various extracellular stimuli (for examples see Ref. 25–28). Such analyses have been possible by taking advantage of DNA microarray assays. In order to determine whether disruption of the YOL002c gene caused global changes in gene expression, we compared the transcriptional response of genes from a yol002c/H9004 strain with an isogenic wild-type strain grown in the presence of myristate. Labeled RNA from each strain was hybridized to Affymetrix S98 Gene-Chips, as described under "Materials and Methods." We screened the resulting data for genes that demonstrated increased expression in yol002c/H9004 cells compared with wild-type cells. This analysis revealed that a significant number of genes whose expression was specifically induced in yol002c/H9004 cells compared with wild-type play a role in phosphate metabolism (Table III). Among the most highly induced genes are PHO5 (5.3-fold) and PHO11 (5.7-fold), both of which are known to be regulated by the PHO pathway that regulates genes involved in phosphate metabolism (29). Thus, YOL002c appears to act as a negative regulator of the PHO system.

In addition, our gene chip analyses revealed that a number of genes involved in fatty acid metabolism were induced in the yol002c strain (for examples see Table III). Taken together, these data suggest that YOL002c plays an important role in cellular metabolism and that cells lacking this protein have multiple defects.

The PHO Signal Transduction Pathway Appears to Be Involved in YOL002c Regulation—The regulation of PHO gene expression by P_i is accomplished via a cascade of events, of which the ultimate regulator is Pho4p, a protein that binds to each regulated PHO gene and activates its transcription. The Pho4p-binding site consists of the following motif: CACGTG and/or CACGTT (30). We identified a putative Pho4p-binding site (CACGTT) in the YOL002c gene promoter 365–370 nucleotides upstream from the initiating ATG codon. When yeast cells are grown under phosphate-rich conditions, Pho4p is phosphorylated by the Pho80p-Pho85p cyclin-cyclin-dependent kinase complex and is exported to the cytoplasm (31, 32). This results in a lack of expression of phosphate-responsive genes. In phosphate-depleted medium, however, Pho4p is localized to the nucleus and actively regulates the expression of PHO genes.

The YOL002c Protein Is Involved in Polyphosphate Accumulation and in Regulation of Acid Phosphatase Activity—Polyphosphate is a linear polymer of up to hundreds of P_i residues linked by phosphoanhydride bonds. In S. cerevisiae, polyphosphate accumulates in vacuoles, and when needed it is hydrolyzed to P_i by an exopolyphosphatase (33). Because disrupting the YOL002c gene appears to affect phosphate metabolism, we asked whether there is any difference in polyphos-
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TABLE III

ORFs that have induced expression in yol002cΔ cells compared with that in wild-type cells, both grown in the presence of myristate

The fold induction from two separate experiments is shown.

| Gene     | Protein name/function                  | Proteins involved in phosphate metabolism | Proteins involved in fatty acid metabolism |
|----------|---------------------------------------|------------------------------------------|------------------------------------------|
| YDR281c  | PHM6-plays role in phosphate metabolism| ×16; ×13.6                                |                                          |
| YBR093c  | PHO5-acid phosphatase                 | ×5.3; ×4.8                                |                                          |
| YAR071c  | PHO11-acid phosphatase                | ×5.7; ×4.2                                |                                          |
| YKL900c  | Type 2B protein phosphatase           | ×2.9; ×3.2                                |                                          |
| YPL019c  | VTC3-polyphosphate synthetase         | ×2.8; ×2.1                                |                                          |
| YJR012c  | VTC4-polyphosphate synthetase         | ×2.8; ×1.6                                |                                          |
| YML133c  | PHO84-inorganic phosphate transporter | ×1.9; ×1.6                                |                                          |
| YBR189c  | PHO89-phosphate permease              | ×1.9; ×1.5                                |                                          |
| YDR060c  | Phosphatidylcholine-sterol O-acyltransferase | ×2.8; ×3.2                      |                                          |
| YLR211c  | Apolipoprotein                         | ×2.7; ×4.7                                |                                          |
| YBR041u  | Fatty acid transporter                 | ×2.2; ×1.7                                |                                          |
| YLR171c  | Phosphatidylethanolamine-binding protein | ×2.1; ×2.6                        |                                          |
| YMR296c  | Serine palmitoyltransferase           | ×1.9; ×1.6                                |                                          |
| YER064c  | Involved with ergosterol-9 expression | ×2.0; ×1.9                                |                                          |
| YIL060c  | Peroxisomal thioloase                  | ×2.0; ×1.9                                |                                          |

![Graph](Image)

**Fig. 5. Expression of YOL002c is reduced when the P concentration in the medium is low.** Relative expression of YOL002c in wild-type cells grown in the absence of phosphate (− phosphate) or in medium containing 10 mM phosphate (+ phosphate). Levels of mRNA for YOL002c were quantitated as described in Fig. 1, taking the expression of cells grown in YPD in the presence of phosphate as 100%.

We proceeded to measure Pi uptake in both wild-type and yol002cΔ cells, and we found that when the cells are grown in medium lacking Pi, 32P is taken up by yol002cΔ cells at a higher rate than in wild-type cells (data not shown). In both wild-type and yol002cΔ cells there is a linear uptake of Pi for at least 20 min when they are incubated in the presence of 0.1 mM phosphate, yol002cΔ cells, however, accumulate approximately twice as much phosphate during this time when compared with the wild-type cells (21 × 10^3 and 13.4 × 10^3 cpm, respectively). Furthermore, acid phosphatase activity is constitutively expressed in yol002cΔ cells even when the cells are grown in phosphate-rich medium, whereas in wild-type cells the activity is repressed in such medium (Fig. 7). The acid phosphatase activity of ydr492wΔ cells was similar to that measured in wild-type cells (Fig. 7), as was that measured in yol101cΔ cells (not shown). The human gene CGI-45 partially rescued the mutant phenotype (Fig. 7).

These data suggest that cells lacking YOL002c are defective in their ability to regulate levels of polyphosphate, and that this defect is partially rescued by the human gene CGI-45.
**DISCUSSION**

We have shown that the YOL002c gene has an ORE in its promoter region and that it is regulated by the Oaf1p/Pip2p transcription factors. However, unlike the majority of genes that are regulated by these proteins, it is not induced by the unsaturated fatty acid oleate but rather is up-regulated in cells grown in the presence of a saturated fatty acid such as myristate. In addition, disruption of the YOL002c gene causes the cells to grow poorly in the presence of myristate, whereas wild-type cells grow at a normal rate on this medium. Furthermore, yol002cΔ cells are resistant to the polycene antibiotic, nystatin. Nystatin resistance has been associated with mutations that lead to changes in the sterols in the cell membrane (24), suggesting that the YOL002c protein may play a role (either direct or indirect) in maintaining the sterol composition of the yeast cell membrane.

By performing gene chip analysis on wild-type cells and yol002cΔ cells grown in the presence of myristate, we found that a number of genes involved in fatty acid metabolism are up-regulated in the mutant strain. In addition, the deletion strain demonstrated increased expression of genes involved in the PHO signaling pathway. There is a putative Pho4p-binding site (CACGTT) in the YOL002c promoter 365–370 nucleotides upstream from the initiating methionine. Genetic studies, together with a recent DNA microarray study, identified more than 20 PHO-regulated genes, most of which contained at least one copy of the Pho4p recognition site (28). Furthermore, this study examined the expression of genes in a strain in which the PHO85 gene, which encodes a cyclin-dependent kinase that interacts with Pho80p to regulate the activity of Pho4p, was disrupted. The data revealed that the mRNA level of YOL002c is decreased under these circumstances (web data from Ref. 28). We found that the expression of YOL002c is decreased when cells are grown in low phosphate media. This observation is consistent with the DNA microarray analysis data obtained for the PHO85 disruption strain and suggests that Pho4p may play a role as a negative regulator of YOL002c.

Phosphate is an essential nutrient that is used in the biosynthesis of many cellular components, including nucleic acids, proteins, lipids, and sugars. The possibility that YOL002c may play a role in the phosphate metabolic pathway led us to compare the amino acid sequence of this protein with that of other proteins involved in this complex pathway. We determined that the carboxyl-terminal portion of YOL002c is homologous to similar regions of Pho80p-like cyclins that are known to interact with the Pho85p cyclin-dependent kinase (34) (Fig. 8). This finding raises the possibility that the multiple phenotypes associated with deleting the YOL002c gene may be mediated through the Pho85p cyclin-dependent kinase, since strains lacking PHO85 have a similar phenotype to yol002cΔ cells.

Based on the published observations described above, and on our recent findings that many genes involved in phosphate metabolism are induced in a strain in which the YOL002c gene is disrupted (Table III), we postulate that YOL002c may act as a negative regulator in the phosphate-dependent signal transduction pathway. This possibility is consistent with our finding that expression of YOL002c is repressed under phosphate-starvation conditions.

We cannot rule out, however, the possibility that the YOL002c protein plays an altogether different role in the cell. For example, a novel positive regulator of PHO5 expression, the PHO23 gene, was recently identified in a genetic screen for PHO81-dependent mutants with a constitutive PHO5 expression phenotype (35). Furthermore, these studies revealed that Pho23p is associated with a histone acetyltransferase activity as well as with the Rpd3p histone deacetylase complex. Rpd3p is the catalytic component of the Rpd3p histone deacetylase complex that also contains Pho23p, Sin3p, Sap30p, and many other proteins (36). Mutations in genes encoding these proteins result in multiple phenotypes including constitutive PHO5 expression, enhanced or derepressed silencing of rDNA, and enhanced silencing of telomeric and mating-type loci (37). Be-
cause deletion of YOL002c results in a strain that is incapable of derepressing acid phosphatase activity (i.e., constitutive PHO5 expression), it is possible that this protein plays a similar role to that of Pho23p. Experiments designed to distinguish between these possible roles of the YOL002c protein are currently in progress.

Our attempts to determine the subcellular location of the YOL002c protein have been unsuccessful thus far. We believe that this is due to instability of the protein. We have attempted to introduce epitope tags within various regions of the protein, as well as at either end, and in each case, we have been unable to detect a product. We are currently in the process of purifying this protein in order to raise specific antibodies, in addition to raising peptide antibodies to hydrophilic portions of the protein. These tools will enable us to determine the subcellular location of YOL002c and, thus will assist us in defining the function of this protein.

We have found that we can partially rescue the defects in phosphate metabolism in the yol002cΔ strain by introducing the human CGI-45 gene into this strain. On the other hand, this human gene failed to rescue the defects associated with fatty acid metabolism in the mutant strain. This raises the possibility that in yeast there is a single gene, YOL002c, that has multifunctional metabolic roles, whereas in humans these various roles may be carried out by different genes. Further analysis of the human genome as the sequence becomes available may reveal additional homologs of YOL002c, whose function is involved in these additional cellular roles.

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