MOLECULAR AND CELLULAR BIOLOGY, Nov. 1998, p. 6560–6570 Vol. 18, No. 11
0270-7306/98/$04.00+0
Copyright © 1998, American Society for Microbiology. All Rights Reserved.

Global Regulatory Functions of Oaf1p and Pip2p (Oaf2p), Transcription Factors That Regulate Genes Encoding Peroxisomal Proteins in *Saccharomyces cerevisiae*

IGOR V. KARPICHEV† AND GILLIAN M. SMALL*

Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, New York, New York 10029

Received 2 June 1998/Returned for modification 14 July 1998/Accepted 28 July 1998

Two transcription factors, Oaf1p and Pip2p (Oaf2p), are key components in the pathway by which several *Saccharomyces cerevisiae* genes encoding peroxisomal proteins are activated in the presence of a fatty acid such as oleate. By searching the *S. cerevisiae* genomic database for the consensus sequence that acts as a target for these transcription factors, we identified 40 genes that contain a putative Oaf1p-Pip2p binding site in their promoter region. Quantitative Northern analysis confirmed that the expression of 22 of the genes identified is induced by oleate and that either one or both of these transcription factors are required for the activation. In addition to known peroxisomal proteins, the regulated genes encode novel peroxisomal proteins, a mitochondrial protein, and proteins of unknown location and function. We demonstrate that Oaf1p regulates certain genes in the absence of Pip2p and that both of these transcription factors play a role in maintaining the glucose-repressed state of one gene. Furthermore, we provide evidence that the defined consensus binding site is not required for the regulation of certain oleate-responsive genes.

In the yeast *Saccharomyces cerevisiae*, the levels of peroxisomal enzymes and the number and size of peroxisomes are increased when the yeast is supplied with a fatty acid carbon source for growth (60). We recently characterized two proteins, Oaf1p and Oaf2p, that act as positive regulators of genes encoding peroxisomal proteins (32, 39). Oaf1p is an oleate-activated transcription factor that was purified through its function of binding to an upstream activating sequence (UAS) in *POX1*, the gene that encodes peroxisomal acyl coenzyme A (acyl-CoA) oxidase in this yeast (39). Binding to this specific DNA sequence results in transcriptional activation of the gene. Using a genetic approach, we identified Oaf2p, a second transcription factor that is also required for the oleate induction of genes encoding peroxisomal proteins (32). The *OAF2* gene was also identified by Rottensteiner et al., who named this gene *PIP2* (46). Deletion of either the *OAF1* or the *PIP2* gene prohibits oleate-induced proliferation of peroxisomes and prevents the yeast from being able to grow on oleate as the sole carbon source. Oaf1p and Pip2p have an overall identity of 40%, with the highest homology occurring in the amino-terminal Zn_2Cys_6 DNA-binding motifs (32). The proteins form a complex and bind to a UAS in the form of a heterodimer (32, 47). DNA sequences to which this heterodimer binds contain palindromic CGG triplets separated by a 15- to 18-nucleotide spacer. This sequence is present in the promoter region of several genes encoding peroxisomal proteins, and it has been termed the oleate response element (ORE) (10, 13).

The *S. cerevisiae* Oaf1p- and Pip2p-dependent pathway that mediates the activation of peroxisomal proteins and peroxisome proliferation resembles an analogous system in higher eukaryotes. In mammals, an increase in peroxisome number and in expression of several peroxisomal enzymes is induced by feeding a high-fat diet (28) or a wide range of compounds that have collectively been termed "peroxisome proliferators" (43). This regulation occurs at the transcriptional level and is controlled by two proteins belonging to the superfamily of nuclear hormone receptors; the peroxisome proliferator-activated receptor (PPAR) and the retinoic acid X receptor (RXR). PPAR and RXR form a heterodimer and bind to DNA elements that contain a direct repeat of the sequence AGG(A/T)CA (57). Three PPAR subtypes have been characterized; PPARδ, which is ubiquitously expressed; PPARα, which is highly expressed in the liver; and PPARγ, which is enriched in adipocytes (50). Activation of PPARγ by 15-deoxyΔ12,14-prostaglandin J2 or a synthetic analog promotes differentiation of preadipocytes into fat cells (16), whereas PPARα mediates the transcriptional effects of drugs that induce peroxisome proliferation (29). Recent studies have shown that various fatty acids and hypolipidemic drugs directly bind to each of the PPARs, but preferentially activate PPARα (9, 14, 33, 35). Furthermore, inhibitors of various steps in the mitochondrial β-oxidation pathway also lead to activation of PPARα and peroxisome proliferation (2, 19, 23). Thus, PPARs appear to act as modulators of lipid homeostasis in higher eukaryotes.

Given the profound phenotypic effect in yeast mutant strains lacking either *OAF1* or *PIP2*, we hypothesized that the Oaf1p and Pip2p transcription factors may play a more global role in regulating genes encoding proteins required for peroxisome function and biogenesis. In order to determine the full extent of the role of these two regulatory proteins, we have taken advantage of the recent completion of the *S. cerevisiae* genome sequencing project (18). We first compared the ORE sequences of eight genes encoding peroxisomal proteins that are known to be induced by oleate and found that only two of the spacer nucleotides are conserved. Based on an ORE consensus sequence derived from these genes, we searched the yeast genome for regions in which this sequence occurs within 500 nucleotides upstream from the initiating codon of an open reading frame (ORF). The expression of genes identified by this search was then measured in wild-type and *oaf1*Δ and *pip2*Δ mutant strains grown in the presence of various carbon

* Corresponding author. Mailing address: Department of Cell Biology and Anatomy, Box 1007, Mount Sinai School of Medicine, New York, NY 10029. Phone: (212) 241-0981. Fax: (212) 860-1174. E-mail: small@msvax.mssm.edu.

† Permanent address: Centre of Bioengineering, Russian Academy of Sciences, Moscow 117312, Russia.

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Reference
W3031A	*MAT***a** *leu2 ura3 trp1 ade2 his3*	34
ΔO1 (*oaf1*Δ)	*MAT***a** *leu2 ura3 trp1* [pRS304-*TRP1-POX1-lacZ*] *ade2 his3 oaf1::HIS3*	42
ΔP2 (*pip2*Δ)	*MAT***a** *leu2 ura3 trp1 ade2 his3 pip2::HIS3*	34
ΔO1P2 (*oaf1*Δ *pip2*Δ)	*MAT***a** *leu2 pip2::LEU2 ura3 trp1 ade2 his3 oaf1::HIS3*	This study
95Δ	*MAT***a** *leu2 ura3 trp1 ade2 his3 YPL095c::HIS3*	This study

sources. Here we describe the role of Oaf1p and Pip2p in the oleate activation of many known peroxisomal membrane and matrix proteins. Furthermore, we demonstrate that these transcription factors are also involved in the regulation of a mitochondrial protein and additional ORFs that encode proteins whose cellular location and function have yet to be defined. Thus far, we have determined that two of these ORFs encode proteins that are localized in peroxisomes.

In this study, we have resolved two different patterns of regulation for oleate-inducible genes. One, previously described for the peroxisomal β-oxidation genes, is Oaf1p and Pip2p dependent and requires the binding of these two proteins to the consensus ORE. A second pattern of regulation does not appear to require the consensus ORE as defined here and can be carried out by either Oaf1p or Pip2p in the absence of the other protein. Whether the transcription factors bind to an ORE or ORE-like sequence in the form of a homodimer or heterodimerize with an additional, as yet unknown, protein or whether activation of the Oaf1p and Pip2p proteins leads to induction of additional transcription factors remains to be established.

MATERIALS AND METHODS

Yeast strains and media. The yeast strains used in this study are described in Table 1. 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), YPG (1% yeast extract, 2% peptone, 3% glycerol), YPGO (0.1% [wt/vol] oleic acid and 0.25% [vol/vol] Tween 40 added to YPG), YPR (1% yeast extract, 2% peptone, 2% raffinose), YPRO (0.1% [wt/vol] oleic acid and 0.25% [vol/vol] Tween 40 added to YPR), or YPE (1% yeast extract, 2% peptone, 2% [wt/vol] ethanol). Auxotrophic supplements were added to 20 μg/ml (40 μg/ml in the case of leucine) as required.

RNA purification and Northern analysis. Strains were precultured in YPD to the mid-logarithmic phase. Precultures were then used to inoculate YPD, YPG, YPGO, YPR, YPRO, or YPE at an optical density at 600 nm of ≈0.1 and grown to the mid-logarithmic phase. Total yeast RNA was isolated as previously described (39). Poly(A)$^+$ fractions were prepared with Oligotex milk as specified by the manufacturer (Quiagene). The mRNA was resolved, transferred to a nylon membrane, and hybridized overnight as described previously (32). Gene-specific probes were generated by PCR amplification with primers based on sequence from the yeast genome database (Stanford) and yeast genomic DNA. The specific primer sequences used are available on request. The PCR products were resolved in a standard 1% agarose gel, purified with a Gene Clean kit (Bio 101), and labeled with a Prime-It RmT kit (Strategene) and [α-^{32}P]dCTP. Following hybridization, the filters were extensively washed and then exposed to a Storage Phosphor Screen. The intensity of each band was determined with Molecular Dynamics Storm 860 software (Imagequant). Values were normalized by using actin (*ACT1*) mRNA expression levels as an internal control for loading. Prior to

TABLE 2. OREs of eight genes encoding peroxisomal proteins that are induced by oleate

Gene	Protein	ORE
POX1	Acyl-CoA oxidase	CGGCTATTAGCCGCTAATGACCG ↑ ↑ 11
FOX2	Hydratase/dehydrogenase	CGGCGTTAACAAATTTCCCCG 9
FOX3	3-Keto acyl-CoA thiolase	CGGGGATAATAGTATTAACACCG 11
SPS19	Peroxisomal 2,4-dienoyl-CoA reductase	CGGCGTTAAGTATATCAAACTCCG 12
CTA1	Catalase	CGGCTTTAACAAATATAAACTCCG 12
PEX11	Peroxisomal membrane protein	CGGCATTTATCTCGGAGAACCCG 11
MDH3	Peroxisomal malate dehydrogenase	CGGAGTTAACGCCGGTCCGGCCG 11
YCAT	Peroxisomal and mitochondrial carnitine acetyltransferase	CGGCTCTGAGAACTAGATACCG 10
Consensus		CGGNNNTNA (N$_{9-12}$)CCG ↑ ↑

[a] Arrows mark the conserved nucleotides.

TABLE 3. Genes in the *S. cerevisiae* genome database that contain a consensus ORE in their promoter regions

Genome	Gene	Protein	Reference(s)
YAR035w	*YAT1*	Putative mitochondrial carnitine acetyltransferase	49
YBR159w	ORF		
YBR297w	*MALC3*	Maltose fermentation regulatory protein	4
YDL078c	*MDH3*	Peroxisomal malate dehydrogenase	41
YDL170w	*UGA3*	Zinc finger protein involved in regulation of the γ-amino-*n*-butyric acid catabolic pathway	1
YDR074w	*TPS2*	Trehalose phosphatase	7
YDR098c	ORF		
YDR244w	*PEX5*	Receptor for PTS1 peroxisomal proteins	58
YDR256c	*CTA1*	Peroxisomal catalase	6
YER015w	*FAA2*	Peroxisomal acyl-CoA synthetase	25, 34
YER018c	ORF		
YFR053c	*HXK1*	Hexokinase I	52
YGL166w	*CUP2*	Transcriptional activator protein of metallothionine	62
YGL205w	*POX1*	Peroxisomal acyl-CoA oxidase	8
YHL027w	*RIM1*	Transcription factor, probable regulator of meiosis	54
YHR015w	ORF		
YIL120w	ORF		
YIL160c	*FOX3*	Peroxisomal thiolase	27
YJL218w	ORF		
YJR019c	*TES1*	Peroxisomal thioesterase	31
YKL171w	ORF		
YKL188c	*PXA2*	Peroxisomal ABC transporter	3, 25
YKR009c	*FOX2*	Peroxisomal hydratase-dehydrogenase	26
YLR284c	ORF		
YLR401c	ORF		
YML042w	*YCAT*	Peroxisomal and mitochondrial carnitine acetyltransferase	11
YNL009w	*IDP3*	Peroxisomal isocitrate dehydrogenase	59
YNL026w	ORF		
YNL035c	ORF		
YNL202w	*SPS19*	Peroxisomal 2,4-dienoyl-CoA reductase	24
YNR001c	*CIT1*	Mitochondrial citrate synthase	55
YOL002c	ORF		
YOL147c	*PEX11*	Peroxisomal membrane protein	12, 40
YOR100c	ORF		
YOR180c	ORF		
YOR184w	*SER1*	Phosphoserine aminotransferase	42
YOR349w	*CIN1*	Gene product affecting microtubule function	53
YOR363c	*PIP2*	Transcription factor involved in peroxisomal proliferation	32, 47
YOR377w	*ATF1*	Alcohol acetyltransferase	17
YPL095c	ORF		

reprobing, the membranes were stripped with 500 ml of 0.2% sodium dodecyl sulfate at 98°C for 30 to 60 min. Expression of each gene was examined in at least two separate mRNA preparations.

YPL095c gene disruption. To disrupt the YPL095c gene, a DNA fragment containing the entire gene was first amplified from yeast genomic DNA. The purified fragment was then subcloned into the PCR 2.1 TA vector, resulting in p95c. This plasmid was digested with *Cla*I, blunt ended, and dephosphorylated, and a 1.7-kb blunt-ended *Bam*HI fragment containing the *S. cerevisiae HIS3* gene was inserted, resulting in p95c::HIS3. The plasmid was digested with *Eco*RI, and the reaction mixture was used for transformation into *S. cerevisiae* W3031A. Selected clones were screened for correct integration by PCR analysis of total DNA isolated from the transformants. A strain carrying a deletion in YPL095c was named 95Δ and was used for further studies.

Other methods. Standard procedures were used for cloning and for transformation of *Escherichia coli* and *S. cerevisiae* cells (48).

RESULTS

Search for genes containing OREs. The Oaf1-Pip2 heterodimer mediates the oleate-dependent induction of *POX1* and *FOX3* by binding to a response element (ORE) present in the promoter regions of these genes (32, 47). Similar elements are found in the promoter regions of other genes encoding peroxisomal proteins in *S. cerevisiae*. By comparing this sequence in eight of these genes, we defined a consensus ORE to consist of the following nucleotides: CGGNNNTNAN$_{9-12}$ CCG (Table 2). With the help of the yeast curator of Stanford University, we carried out a search for the occurrence of this sequence within the yeast genome. In total, the consensus sequence occurs approximately 200 times. By selecting those genes or ORFs in which the ORE is located within 500 nucleotides upstream from the initiation codon, we identified the 40 genes or ORFs that are shown in Table 3.

Induction of known peroxisomal proteins. Thirteen of the genes shown in Table 3 encode known peroxisomal proteins (shown in boldface). As predicted, these include the eight genes shown in Table 2. Several of the genes identified have previously been shown to be induced by oleate, and in some cases, Oaf1p and/or Pip2p has been implicated in their regulation (10, 13, 24, 31, 46, 59, 61). We examined the expression of each of these genes in a wild-type yeast strain and in *oaf1*Δ and *pip2*Δ null mutants, as well as in a strain carrying deletions in both of these genes. The strains were each grown in the presence of glucose, glycerol, or glycerol and oleate.

Our results demonstrated that the three genes encoding peroxisomal β-oxidation enzymes (*POX1*, *FOX2*, and *FOX3*) are expressed and regulated in an identical fashion. Each transcript is repressed by growth in glucose medium, derepressed when the cells are grown in the presence of glycerol, and activated approximately 10-fold by growth in glycerol-oleate medium. The induction by oleate, but not the derepression in

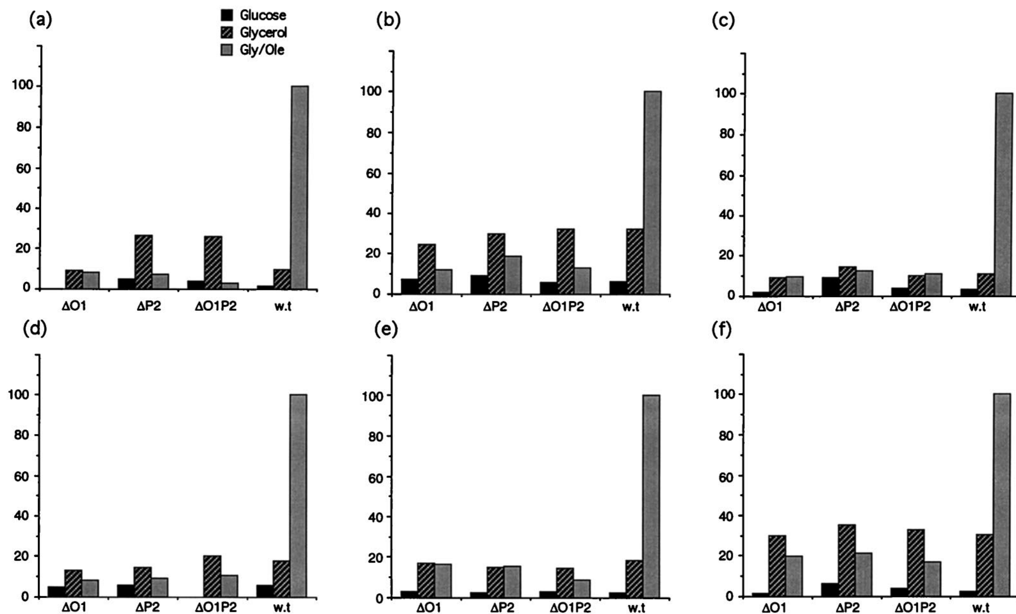

FIG. 1. Expression of genes encoding peroxisomal proteins in wild-type (w.t.), *oaf1*Δ (ΔO1), *pip2*Δ (ΔP2), and *oaf1*Δ *pip2*Δ (ΔO1P2) yeast strains. Poly(A)$^{+}$ RNA fractions from cells grown in 50-ml cultures were resolved in a 1% formaldehyde agarose gel (see Materials and Methods). Cells were grown either in glucose (YPD), glycerol (YPG), or glycerol-oleate (YPGO) medium. Levels of mRNA for *POX1* (YGL20Sw) (a), *FOX2* (YKR009c) (b), *FOX3* (YIL160c) (c), *TES1* (YJR019c) (d), *SPS19* (YNL202w) (e), and *PEX11* (YOL147w) (f) were quantitated from a Northern blot, and the values were normalized with actin levels as an internal control for loading. Expression in our wild-type strain grown in the presence of oleate was taken to be 100%.

glycerol, requires Oaf1p and Pip2p (Fig. 1a to c). The peroxisomal proteins encoded by *TES1* (31), *SPS19* (24), and *PEX11* (12, 40) are regulated in the same manner as the β-oxidation genes (Fig. 1d to f).

A different pattern of regulation was seen for *FAA2*, *PEX5*, *MDH3*, *PXA2*, *YCAT*, and *IDP3*, genes that encode peroxisomal matrix and membrane proteins. Each of these genes is repressed by growth in glucose to variable extents and is only moderately induced by oleate (two- to threefold) in our wild-type strain (Fig. 2). In the *oaf1*Δ and *pip2*Δ strains grown in the presence of oleate, expression of each of these transcripts is marginally higher than that in glycerol-grown cells (with the exception of *MDH3* in *oaf1*Δ). However, in a strain carrying disruptions of both *OAF1* and *PIP2* (ΔO1P2), the expression of

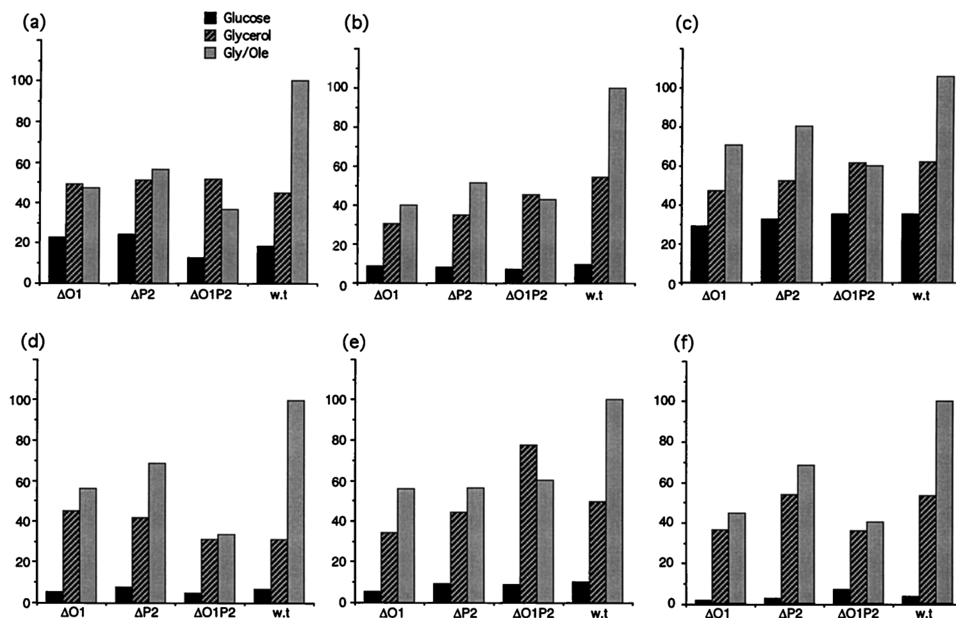

FIG. 2. Expression of the six genes, *MDH3* (YDL078c) (a), *PEX5* (YDR244w) (b), *FAA2* (YER015w), (c), *PXA2* (YKL188c) (d), *YCAT* (YML042w) (e), and *IDP3* (YNL009w) (f), that encode peroxisomal membrane or matrix proteins is induced two- to threefold in glycerol- and oleate (Gly/Ole)-grown cells. The induction is completely abolished when the *OAF1* and *PIP2* genes are both disrupted, but there is partial induction in cells lacking only one of these genes. w.t., wild type. Levels of expression were quantitated and normalized as described in the legend to Fig. 1.

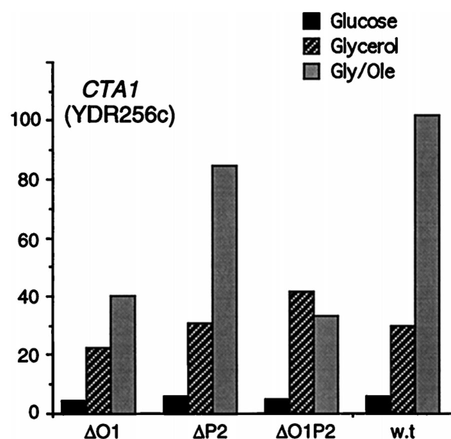

FIG. 3. Oleate-mediated induction of peroxisomal catalase requires Oaf1p but not Pip2p. w.t., wild type; Gly/Ole, glycerol and oleate. *CTA1* mRNA expression was measured and quantitated as described in the legend to Fig. 1.

these genes in glycerol-oleate-grown cells is equal to or lower than the expression in cells grown in glycerol (Fig. 2).

Regulation of *CTA1*, the gene encoding peroxisomal catalase, appears to be unique among the genes described here. This gene is induced approximately fivefold in glycerol-oleate-grown cells compared to the expression in cells grown in glycerol alone, and the induction is dependent on Oaf1p but not Pip2p (Fig. 3).

Regulatory proteins. Five of the remaining genes that are listed in Table 3 (*MAL33*, *UGA3*, *CUP2*, *RIM1*, and *PIP2*) encode regulatory proteins. We and others have previously demonstrated that Pip2p (Oaf2p) is induced by oleate (32, 47). To determine whether induction of the *PIP2* transcript requires the presence of *OAF1*, we performed a Northern analysis of total RNA isolated from our wild-type strain and from the *oaf1*Δ and *pip2*Δ strains. Our results clearly show increased expression of *PIP2* mRNA in cells grown in the presence of oleate, and this induction is abolished in the absence of *OAF1* (Fig. 4). These findings are in agreement with those from a previous report demonstrating that β-galactosidase activity expressed from a *PIP2-lacZ* reporter construct was induced by oleate, and this induction was absent in an *oaf1*Δ strain (47).

Expression of the remaining genes in Table 3 that encode regulatory proteins was not induced by growth in the presence of oleate and was no different in the *oaf1*Δ and *pip2*Δ strains compared to that in our wild-type strain (data not shown).

Genes encoding proteins with known function. Seven of the genes in Table 3 encode nonperoxisomal proteins of known

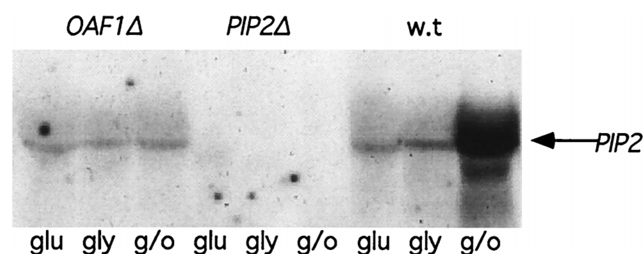

FIG. 4. Northern analysis of *PIP2* expression in a wild-type strain (w.t.) and strains in which *OAF1* or *PIP2* have been disrupted. Sixty micrograms of total RNA isolated from cells grown in YPD (glu), YPG (gly), or YPGO (g/o) medium was resolved in a 1% formaldehyde agarose gel, transferred to a nylon membrane, and hybridized as described in Materials and Methods.

TABLE 4. Oleate induction of transcripts expressed from ORFs that have an ORE consensus sequence and requirement for *OAF1* and/or *PIP2*

ORF	Approx fold induction on YPGO vs YPG	Requirement for regulation[a]	
		OAF1	*PIP2*
YBR159w	2	+	+
YDR098c		−	−
YER018c		−	−
YHR015w		−	−
YIL120w	2	+	−
YJL218w	10	+	+
YKL171w		−	−
YLR284c	10	+	+
YLR401c		−	−
YNL026w		−	−
YNL035c		−	−
YOL002c		+	+
YOR100c	2	+	+
YOR180c	10	+	+
YPL095c	10	+	+

[a] +, gene required; −, gene not required.

function. These include *YAT1*, encoding a mitochondrial acetyltransferase (49); *TPS2*, encoding trehalose phosphatase (7); *HXK1*, encoding hexokinase 1 (52); *SER1*, encoding serine aminotransferase (42); *ATF1*, encoding alcohol acetyltransferase (17); *CIN1*, encoding a protein required for microtubule stability (53); and *CIT1*, encoding citrate synthase (55). Of these, only the *CIT1* transcript was induced by oleate and regulated by Oaf1p and Pip2p (Fig. 5a). This result was somewhat surprising in light of the fact that *CIT1* encodes a mitochondrial isoform of citrate synthase, whereas the *CIT2* gene encodes a peroxisomal isoform (36, 45, 55). Increased transcription of *CIT2*, via a retrograde mechanism of regulation involving the *RTG* genes, occurs when mitochondrial function is impaired (5, 37, 38). Interestingly, these studies also demonstrated that the *RTG* genes are required for oleate-dependent peroxisome proliferation and that transcription of *CIT2* is increased in an *RTG1*- and *RTG2*-dependent manner in cells grown in raffinose-oleate media (5). In order to determine whether Oaf1p and Pip2p are also involved in the regulation of *CIT2*, we compared the expression of this gene in wild-type and *oaf1*Δ *pip2*Δ strains grown in several different media. *CIT2* is expressed at low levels in YPD-, YPG-, and YPGO-grown wild-type cells and at higher levels in cells grown in YPR. The expression is elevated when oleate is added to the raffinose medium (Fig. 5b). In the *oaf1*Δ *pip2*Δ double disruption strain, *CIT2* is expressed at similar levels to the wild-type strain in all media tested. Thus, in contrast to the mode of *CIT1* regulation, Oaf1p and Pip2p appear to play no role in the oleate-dependent induction of *CIT2*.

Our finding that *CIT1* is induced by oleate in an Oaf1p- and Pip2p-dependent manner demonstrates that regulation by these two transcription factors is not restricted to genes required for peroxisome biogenesis or function.

ORFs. Our search for ORE-containing genes retrieved 15 ORFs that contain this sequence in their promoter regions. Seven of these genes are induced in cells grown in the presence of oleate via pathways that require Oaf1p or the Oaf1p-Pip2p heterodimer (Table 4).

(i) YLR284c and YOR180c. We noted that two of the ORFs shown in Table 4 (YLR284c and YOR180c) encode putative proteins that contain amino acid sequence motifs similar to the

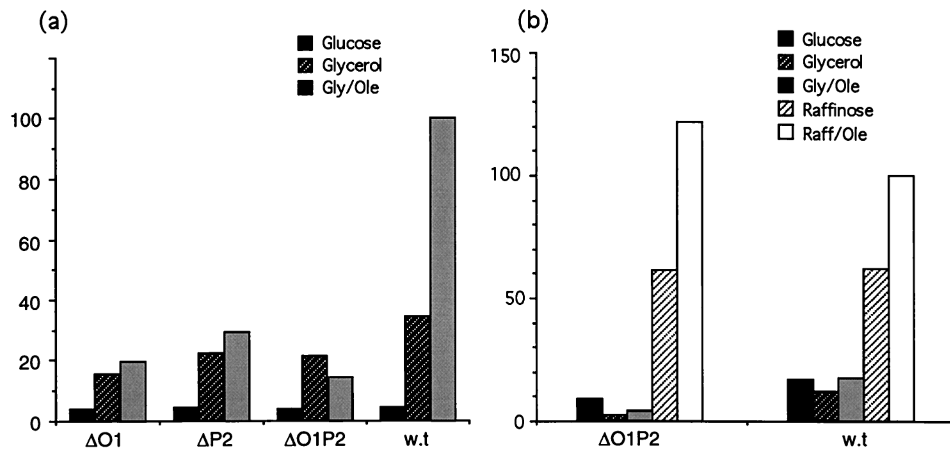

FIG. 5. Oleate-dependent induction of genes encoding two different isoforms of citrate synthase. (a) *CIT1* (YNR001c), encoding mitochondrial citrate synthase, is induced by oleate and is regulated by Oaf1p and Pip2p. (b) The oleate-dependent induction of *CIT2* (YCR005c), encoding a peroxisomal isoform of this enzyme, is not mediated by these transcription factors. Growth media and method of quantitation are described in the legend to Fig. 1, except that cells were also grown in raffinose (YPR) or raffinose-oleate (YPRO [Raff/Ole]) medium for the analysis of *CIT2* expression.

peroxisomal targeting signals 1 and 2 (PTS1 and PTS2, respectively) (20, 21). The transcripts for both of these ORFs are induced in the presence of oleate in a fashion similar to that of the genes encoding the peroxisomal β-oxidation enzymes, and this induction requires the presence of Oaf1p and Pip2p (data not shown). We have subsequently demonstrated that these ORFs encode novel peroxisomal proteins belonging to the enoyl-CoA hydratase family (unpublished data).

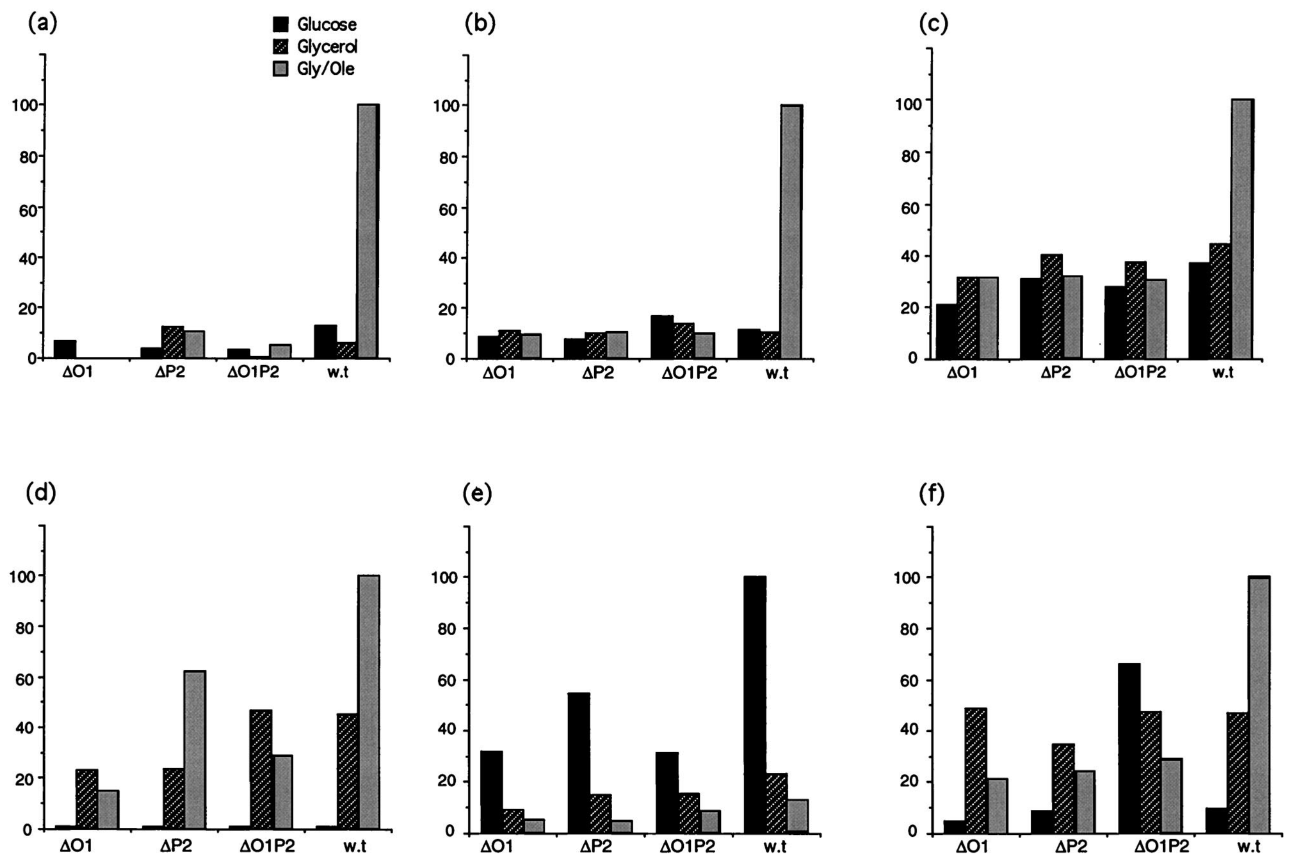

FIG. 6. mRNA expression of the ORFs that are regulated by Oaf1p and/or the Oaf1p Pip2p heterodimer. YJL218w (a) and YPL095c (b) are induced by oleate and regulated by Oaf1p and Pip2p in a similar fashion to genes encoding peroxisomal β-oxidation enzymes (Fig. 1). YBR159w (c) is induced by oleate and regulated by Oaf1p and Pip2p, whereas partial induction of YIL120w (d) is mediated by Oaf1p alone. High expression of YOL002c (e) in the presence of glucose is abolished in *oaf1*Δ and *pip2*Δ strains. The oleate-dependent induction of YOR100c (f) requires both Oaf1p and Pip2p, whereas glucose repression of this gene is abolished in an *oaf1*Δ *pip2*Δ strain. w.t., wild type; Gly/Ole, glycerol and oleate. Growth media and method of quantitation are described in the legend to Fig. 1.

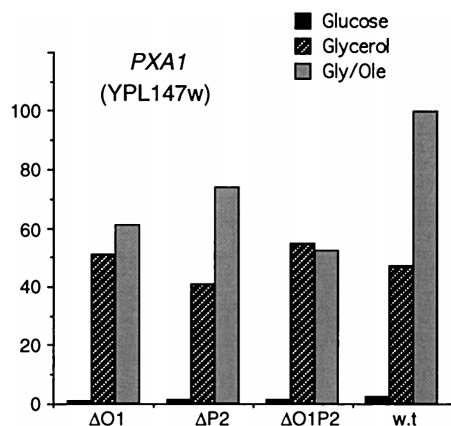

FIG. 7. *PXA1*, encoding a peroxisomal membrane protein, lacks a consensus ORE in its promoter region, but is induced by oleate in an Oaf1p- and Pip2p-dependent fashion. w.t., wild type; Gly/Ole, glycerol and oleate. Expression was quantitated and normalized as described in the legend to Fig. 1.

(ii) YPL095c and YJL218w. The transcripts expressed by YJL218w and YPL095c are also induced approximately 10-fold when cells are grown in the presence of oleate and are expressed at low levels in both glucose- and glycerol-grown cells (Fig. 6a and 6b).

The YJL218w ORF encodes a putative 21.5-kDa protein predicted to contain at least one transmembrane domain. It has no close homolog in *S. cerevisiae*, but has similarity to galactoside *O*-acetyltransferase from *E. coli*. Experiments are in progress to determine the localization and function of the YJL218w protein.

The YPL095c ORF encodes a protein of 456 amino acids that has a predicted molecular mass of 51.7 kDa and an isoelectric point of 7.61. This protein has no close homology to any known protein family; however, a database search revealed a close yeast homolog encoded by the YBR177c ORF. The YPL095c protein contains no obvious localization signal. As a first step toward determining the function of this protein, the genomic copy of YPLO95c was replaced with *HIS3*, yielding the null mutant strain 95Δ (see Materials and Methods). 95Δ cells fail to grow on a nonfermentable carbon source (glycerol or ethanol), suggesting that mitochondrial respiration may be impaired in these cells. Experiments are in progress to further define the role of the YPL095c protein.

(iii) YBR159w, YIL120w, YOR100c, and YOL002c. The remaining ORFs that are regulated by the Oaf1 and Pip2 proteins are each predicted to encode membrane proteins. The expression and regulation of each of these transcripts are somewhat different. YBR159w is expressed at approximately equal levels in glucose- and glycerol-grown cells and is induced two- to threefold in cells grown in the presence of oleate. This induction requires both Oaf1p and Pip2p (Fig. 6c).

The YIL120w ORF encodes a protein that contains 12 regions predicted to be transmembrane domains. The gene is expressed at very low levels in the presence of glucose and is induced approximately twofold by growth in glycerol-oleate compared to that in glycerol alone. This induction is abolished in an *oaf1*Δ strain, but it is still evident, although reduced, in a *pip2*Δ strain. Thus, regulation of this gene resembles the mode of *CTA1* regulation (compare Fig. 6d with Fig. 3).

The YOL002c ORF is predicted to encode a protein that contains seven transmembrane domains. In our wild-type yeast strain, the YOL002c transcript is highly expressed in the presence of glucose and is expressed at lower levels in cells grown in glycerol or glycerol and oleate. In the absence of Oaf1p or Pip2p, the expression of this transcript is reduced in each growth medium tested (Fig. 6e). These data suggest that the role of Oaf1p and Pip2p is not restricted to activating oleate-responsive genes, but appears to be more complex.

The YOR100c ORF is predicted to encode a protein of 327 amino acids that has similarity to mitochondrial carnitine acyltransferase proteins. While expression of this transcript is repressed by glucose in wild-type and *oaf1p*Δ and *pip2p*Δ strains, it is not repressed in a strain carrying disruptions in both of these genes (Fig. 6f). This result further supports the idea that the function of Oaf1p and Pip2p is not restricted to mediating oleate-dependent induction, because they appear to also play a role in maintaining the glucose-repressed state of this gene.

Oleate-dependent induction of *PXA1*, a gene that does not contain the consensus ORE. Pat1p and Pat2p are peroxisomal membrane proteins that comprise the two halves of an ABC transporter required for the import of long-chain fatty acids into peroxisomes (25). The proteins are encoded by the *PXA2* and *PXA1* (*PAL1*) genes, respectively (51, 56). Both proteins are reported to be induced by oleate; however, while *PXA2* contains a consensus ORE, *PXA1* lacks this binding sequence in its promoter region. To determine whether the regulation of the Pat2 protein requires Oaf1p or Pip2p, we examined the expression of the *PXA1* transcript in our wild-type and *oaf1 pip2* null strains. We found that *PXA1* is induced approximately twofold in the presence of oleate, and this induction is abolished in the *oaf1*Δ *pip2*Δ strain (Fig. 7). Thus, moderate increases in *PXA1* transcription in cells grown in the presence of oleate resemble the increased expression of the genes described in Fig. 2. These data suggest that while such regulation requires either Oaf1p or Pip2p, it differs from the mechanism by which highly induced peroxisomal proteins (e.g., β-oxidation enzymes) are activated, since it does not necessarily require a consensus ORE.

DISCUSSION

We have taken advantage of the completed yeast genome database to search for genes containing a consensus binding site for the transcription factors that are responsible for mediating induced expression of genes encoding peroxisomal proteins. This search identified 40 genes that have oleate response-like elements in their promoter regions (Table 3). Twenty-two of the transcripts expressed from these genes are induced in cells grown in the presence of oleate, under the control of Oaf1p and Pip2p. These include genes encoding known, as well as newly identified, peroxisomal proteins in addition to genes encoding a mitochondrial protein and proteins of unknown location and function. mRNA expression of 11 of these genes was approximately 10-fold higher in glycerol-oleate-grown cells than that in cells grown in glycerol alone, whereas induction of the remaining genes was only 2- to 3-fold.

The OREs in the regulated genes are localized on either the Watson or the Crick DNA strand, and they are not identical (Table 5). However, nucleotides at certain positions appear to be conserved. Following the first CGG, positions 1 and 2 are variable, position 3 is A/T, position 4 is T, position 5 is A/T, and position 6 is A. Nucleotides at all other positions are totally random, except for those at positions 15 and 17, which are A, T, or C. There are four regulated genes with OREs that do not conform to this consensus (footnote b in Table 5). It is possible that there are additional ORE-like elements that act as targets for the Oaf1-Pip2 heterodimer that would not be

TABLE 5. OREs of genes shown in Table 3

Gene or ORF	Sequence[a]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Regulated by Oaf1p and Pip2p																		
POX1	C	T	A	T	T	A	G	C	C	G	C	T	A	A	T	G	A	
FOX2	C	G	T	T	A	A	C	A	A	A	T	T	T	C	C			
FOX3	G	G	A	T	A	A	T	A	G	T	A	T	T	A	A	C	A	
PEX11	C	A	T	T	T	A	T	C	T	C	G	G	A	G	A	A	C	
TES1	T	G	T	T	T	A	T	G	C	C	G	T	C	T	C	T	T	
YLR284c	G	C	A	T	A	A	G	A	A	A	T	A	T	T	A	T	T	
YOR180c	A	G	T	T	T	A	G	C	G	C	G	C	T	T	A	C	A	G
YPL095C	C	T	T	T	A	A	T	A	T	A	C	T	T	T	C	A		
YJL218W	C	C	A	T	A	A	C	G	C	T	A	T	T	T	C	T		
CIT1[b]	A	A	A	T	**G**	A	A	A	A	G	T	A	T	G	A	C	C	C
YBR159W	A	C	T	T	A	A	A	A	C	A	G	A	A	C	A	T		
MDH3[b]	A	G	T	T	A	A	C	G	C	C	G	G	T	C	C	G	**G**	
PEX5	A	G	T	T	A	A	T	C	G	C	G	A	T	G	A	T		
CTA1	C	T	T	T	A	A	C	A	A	A	T	A	T	A	A	A	C	T
YIL120W[b]	G	T	T	T	**C**	A	T	G	T	T	T	C	T	G	**G**	C		
YCAT	C	T	**C**	T	**G**	A	G	A	A	C	T	A	G	A	T	A		
IDP3	C	A	T	T	A	A	G	C	A	T	A	C	T	A	A	A	T	
YOR100C	A	T	A	T	T	A	G	G	A	G	C	A	G	T	A	A	C	
SPS19	C	G	T	T	A	A	G	T	A	T	A	T	C	A	A	A	C	T
PIP2[b]	A	A	**C**	T	A	A	A	T	A	T	A	A	T	C	T			
PXA2	C	G	T	T	A	A	T	T	A	C	A	A	C	T	A	T	C	A
YOL002C	G	G	T	T	A	A	T	A	G	G	C	G	G	T	C	T	T	
Consensus	N	N	T/A	T	T/A	A	N	N	N	N	N	N	N	N	T/A/C	N	T/A/C	N
Not regulated by Oaf1p or Pip2p																		
FAA2[b]	C	G	T	T	A	A	A	G	A	C	T	G	A	C	**G**	G	T	
YAT1[b]	G	G	A	T	**G**	A	C	C	G	C	G	G	A	C	C	G		
MAL33	A	T	T	T	T	A	T	T	G	T	C	C	G	T	A			
UGA3	T	T	T	T	A	A	T	G	C	G	T	C	A	A	T			
TPS2[b]	C	A	**G**	T	**G**	A	G	A	C	G	G	A	G	C	**G**	G		
YDR098C[b]	T	T	T	T	**G**	A	T	C	C	G	G	A	C	A	A	C	C	
YER018C[b]	A	C	A	T	A	A	T	T	G	C	G	T	T	G	**G**	T	C	
HXK1[b]	T	C	**C**	T	A	A	G	G	T	A	C	C	A	A	A	A	T	
CUP2[b]	C	T	**C**	T	T	A	C	C	C	T	G	C	G	T	**G**	C		
RIM1[b]	A	C	**G**	T	A	A	T	T	T	T	A	G	A	A	A	C	T	
YHR015W[b]	T	T	T	T	**G**	A	T	C	C	G	G	A	C	A	A	C	C	
YKL171W[b]	C	G	**C**	T	A	A	T	A	T	T	A	A	T	G	**G**	C		
YLR401C	C	A	T	T	A	A	G	T	C	T	C	T	T	C	A	T	C	G
YNL026W[b]	G	C	**G**	T	A	A	A	A	A	C	T	A	T	T	A	A	T	
YNL035C[b]	G	G	A	T	**C**	A	G	C	G	G	T	T	C	G	A	T	C	
CIN1[b]	A	G	A	T	**C**	A	A	A	T	A	C	C	G	G	**G**	C		
ATF1[b]	T	T	T	T	T	A	T	A	T	T	T	T	T	T	**G**			
SER1	C	A	T	T	A	A	T	T	T	A	C	C	T	G	A	C	A	

[a] Numbers 1 to 18 represent the nucleotide position in the spacer sequence separating the CGG triplets.
[b] Gene does not conform to the consensus sequence. Aberrant nucleotide(s) is in boldface.

retrieved in our search. For example, there may be some variability at positions 4 and 6, or the number of nucleotides in the spacer may be less than 9 or greater than 12. Nevertheless, the defined consensus ORE sequence that we used in this search enabled us to identify 13 genes encoding known peroxisomal proteins, as well as at least two ORFs that encode novel peroxisomal proteins, all of which are induced by oleate and regulated either by Oaf1p or by the Oaf1p-Pip2p heterodimer.

Four of the 18 genes that are not regulated by Oaf1p and Pip2p contain putative OREs that conform to the consensus sequence described above (Table 5). This finding, together with the fact that certain regulated genes do not contain the consensus ORE as defined here (e.g., *PXA1*), implies that there must be additional factors that determine whether this sequence acts as a functional regulatory element. It is likely that the flanking sequences around the putative ORE influences the selectivity or strength of Oaf1p and Pip2p binding. The fact that the nucleotides in the flanking region upstream of the PPRE (the binding site for the PPAR-RXR heterodimer) play an important role in the strength of binding, and thus in the level of transcriptional activation of PPAR-regulated genes (30), supports this hypothesis. In our own experiments we have found that the flanking regions of the *POX1* ORE are crucial for its activity, since the introduction of *Xho*I sites on either side of the ORE abolished its function (unpublished results).

Several peroxisomal proteins that are reported to be induced by oleate are encoded by genes that do not contain an obvious ORE consensus sequence. One such example is the *PXA1* gene (also called *PAL1*), which encodes Pat2p, a peroxisomal ATP-binding cassette transporter protein (51, 56). β-Galactosidase

activity expressed from a *PXA1-LacZ* fusion is induced 10-fold in cells grown in the presence of oleate compared to that in cells grown in glycerol (56). Our results show that expression of *PXA1* in cells grown in the presence of oleate is approximately twofold greater than that in cells grown in glycerol and appears to be regulated in a similar manner to those of several other genes encoding peroxisomal proteins that do contain the consensus ORE (compare Fig. 7 with Fig. 2). This finding raises the possibility that the consensus ORE defined here is only critical in those genes that are highly induced by oleate (10-fold) and that require both Oaf1p and Pip2p for their induction. The manner in which the genes that are marginally induced by oleate are regulated remains unclear. For these genes, the presence of Oaf1p or Pip2p alone appears to be sufficient for induced expression by oleate, whereas in the absence of both of these proteins, the induction is abolished. Whether the transcription factors bind to the ORE or an ORE-like sequence or whether they bind to an alternative, as yet unidentified, DNA sequence remains to be determined. A further possibility to consider is that activation of the Oaf1 and Pip2 proteins may lead to induction of an additional factor(s) that may bind to a different *cis*-acting element in the *PXA1* promoter and thus mediate transcriptional activation.

The manner in which *CTA1*, the gene encoding peroxisomal catalase, is regulated differs from that of the other genes encoding peroxisomal proteins described thus far. Oleate-dependent induction of *CTA1* requires Oaf1p, but not Pip2p (Fig. 3). This finding is in agreement with our previous data obtained with a strain carrying a mutation in the *PIP2* (*OAF2*) gene (32). It has also been demonstrated that catalase A activity in oleate-grown cells is higher in a *pip2*Δ strain than in an *oaf1*Δ or *oaf1*Δ *pip2*Δ strain (47). Together these results suggest that Oaf1p alone is able to mediate the transcriptional activation of *CTA1*, perhaps in the form of a homodimer or complexed with additional as yet unidentified protein(s). The presence of Pip2p appears to enhance this induction. YIL120w, one of the ORFs identified by our search, is regulated in a fashion similar to that of the *CTA1* transcript (see below).

In this study, we demonstrated that expression of the gene encoding mitochondrial citrate synthase is stimulated by the Oaf1-Pip2 heterodimer in response to oleate. To our knowledge, this is the first example in yeast in which the transcription factors required for peroxisome proliferation also regulate expression of a mitochondrial protein. However, such examples have been reported in higher eukaryotes. The PPAR-RXR heterodimer mediates induction of mitochondrial acyl-CoA dehydrogenase in rat liver and in cultured cells when the enzyme carnitine palmitoyl transferase I (CPT-I) is inhibited (23). In addition, PPAR and RXR activate the mitochondrial 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase gene in response to the hypolipidemic drug clofibrate or to fatty acids, such as linoleic acid and oleic acid (44). Thus, these mammalian transcription factors play a more global role in regulating lipid metabolism than was initially recognized.

By implementing the approach described here to identify Oaf1p- and Pip2p-regulated genes, we retrieved seven novel ORFs that are induced by oleate. It is now of great interest for us to determine the subcellular location and function of these ORFs. Thus far, we have demonstrated that YLR284c and YOL180c encode novel peroxisomal proteins (unpublished data). Furthermore, our initial results suggest that the YPL095c protein is required for normal mitochondrial function.

In addition to their role in activation of oleate-responsive genes, we obtained evidence that Oaf1p and Pip2p are also involved in maintaining the inactive state of the glucose-repressed YOR100c gene. In contrast to the requirement of both of these proteins for oleate-dependent transcriptional activation of this gene, the presence of either Oaf1p or Pip2p is sufficient for maintaining its glucose-repressed state. One possible explanation for this mode of regulation would postulate that either one of these proteins is capable of binding, either as a homodimer or complexed with an additional factor(s), to a repression element in the YOR100c promoter when cells are grown in the presence of glucose. When the cells are shifted to an oleate medium, Pip2p is induced, which may in turn cause the Oaf1p-Pip2p heterodimer to predominate and bind to the ORE, thus activating transcription of the gene. Alternatively, regulation of YOR100c may represent an example in which transcription factors can act as repressors or activators while bound to the same regulatory element, a phenomenon that has been described for some members of the steroid hormone superfamily of nuclear receptors. For example, heterodimers of the thyroid hormone receptor and the retinoic acid receptor can activate or repress transcription of target genes, depending on the presence or absence of ligand (15, 22). It was postulated that negative control in the absence of ligand would greatly increase the specificity of gene regulation for response elements with overlapping receptor specificity (22).

In summary, our search for genes containing a consensus DNA-binding site for the Oaf1p-Pip2p heterodimer has led to the identification of 22 genes that are regulated by one or both of these transcription factors. In addition, subsequent experiments have revealed that the mechanism by which Oaf1p and Pip2p mediate transcriptional regulation depends on the nature of the target gene.

ACKNOWLEDGMENTS

We acknowledge and thank Murl Casey for excellent technical assistance. We also extend thanks to the yeast curator at Stanford University for assistance with the yeast genome database search and to Joel Lopez for assistance with the Northern analysis of YOL002c.

This research was supported by American Heart Association grants 95008910 and 92001690 and by NIH grant R55DKOD51992.

REFERENCES

1. **Andre, B.** 1990. The *UGA3* gene regulating the GABA catabolic pathway in *Saccharomyces cerevisiae* codes for a putative zinc-finger protein acting on RNA amount. Mol. Gen. Genet. **220:**269–276.
2. **Asiedu, D. K., J. Skorve, N. Willumsen, A. Demoz, and R. K. Berge.** 1993. Early effects on mitochondrial and peroxisomal β-oxidation by the hypolipidemic 3-thia-fatty acids in rat livers. Biochim. Biophys. Acta **1166:**73–76.
3. **Bossier, P., L. Fernandes, C. Vilela, and C. Rodrigues-Pousada.** 1994. The yeast YKL741 gene situated on the left arm of chromosome XI codes for a homologue of the human ALD protein. Yeast **10:**681–686.
4. **Charron, M. J., E. Read, S. R. Haut, and C. A. Michels.** 1989. Molecular evolution of the telomere-associated *MAL* loci of *Saccharomyces*. Genetics **122:**307–316.
5. **Chelstowska, A., and R. A. Butow.** 1995. *RTG* genes in yeast that function in communication between mitochondria and the nucleus are also required for expression of genes encoding peroxisomal proteins. J. Biol. Chem. **270:** 18141–18148.
6. **Cohen, G., F. Fessl, A. Traczyk, J. Rytka, and H. Ruis.** 1985. Isolation of the catalase A gene of *Saccharomyces cerevisiae* by complementation of the cta1 mutation. Mol. Gen. Genet. **200:**74–79.
7. **De Virgilio, C., N. Burckert, W. Bell, P. Jeno, and A. Wiemken.** 1993. Disruption of *TPS2*, the gene encoding the 100-kDa subunit of the trehalose-6-phosphate synthase/phosphatase complex in *Saccharomyces cerevisiae*, causes accumulation of trehalose-6-phosphate and loss of trehalose-6-phosphate phosphatase activity. Eur. J. Biochem. **212:**315–323.
8. **Dmochowska, A., D. Dignard, R. Maleszka, and D. Y. Thomas.** 1990. Structure and transcriptional control of the *Saccharomyces cerevisiae POX1* gene encoding acyl-coenzyme A oxidase. Gene **88:**247–252.
9. **Dowell, P., V. Peterson, J. T. M. Zabriskie, and M. Leid.** 1997. Ligand-induced peroxisome proliferator-activated receptor α conformational change. J. Biol. Chem. **272:**2013–2020.
10. **Einerhand, A. W. C., W. T. Kos, B. Distel, and H. F. Tabak.** 1993. Characterization of a transcriptional control element involved in proliferation of

peroxisomes in yeast in response to oleate. Eur. J. Biochem. **314:**323–331.
11. **Elgersma, Y., W. T. van Roermund, R. J. A. Wanders, and H. F. Tabak.** 1995. Peroxisomal and mitochondrial carnitine acetyltransferase of *Saccharomyces cerevisiae* are encoded by a single gene. EMBO J. **14:**3472–3479.
12. **Erdmann, R., and G. Blobel.** 1995. Giant peroxisomes in oleic acid-induced *Saccharomyces cerevisiae* lacking the peroxisomal membrane protein Pmp27p. J. Cell Biol. **128:**509–523.
13. **Filipits, M., M. M. Simon, W. Rapatz, B. Hamilton, and H. Ruis.** 1993. A *Saccharomyces cerevisiae* upstream activating sequence mediates induction of peroxisome proliferation by fatty acids. Gene **132:**49–55.
14. **Forman, B. M., J. Chen, and R. M. Evans.** 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. Proc. Natl. Acad. Sci. USA **94:**4312–4317.
15. **Forman, B. M., and H. H. Samuels.** 1990. Dimerization among nuclear hormone receptors. New Biol. **2:**587–594.
16. **Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans.** 1995. 15-Deoxy-delta 12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell **83:**803–812.
17. **Fujii, T., N. Nagasawa, A. Iwamatsu, T. Bogaki, Y. Tamai, and M. Hamachi.** 1994. Molecular cloning, sequence analysis, and expression of the yeast alcohol acetyltransferase gene. Appl. Envir. Microbiol. **60:**2786–2792.
18. **Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, E. J. Louis, H. W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin, and S. G. Oliver.** 1996. Life with 6000 genes. Science **274:**562–567.
19. **Gottlicher, M., A. Demoz, D. Svensson, P. Tollet, R. K. Berge, and J. A. Gustafsson.** 1993. Structural and metabolic requirements for activators of the peroxisome proliferator-activated receptor. Biochem. Pharamacol. **46:**2177–2184.
20. **Gould, S. J., G.-A. Keller, N. Hosken, J. Wilkinson, and S. Subramani.** 1989. A conserved tripeptide sorts proteins to peroxisomes. J. Cell Biol. **108:**1657–1664.
21. **Gould, S. J., G.-A. Keller, and S. Subramani.** 1988. Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. J. Cell Biol. **107:**897–905.
22. **Graupner, G., K. N. Wills, M. Tzukerman, X.-K. Zhang, and M. Pfahl.** 1989. Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity. Nature **340:**653–656.
23. **Gulick, T., s. Cresci, T. Caira, D. D. Moore, and D. P. Kelly.** 1994. The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. Proc. Natl. Acad. Sci. USA **91:**11012–11016.
24. **Gurvitz, A., H. Rottensteiner, S. H. Kilpelainen, A. Hartig, J. K. Hiltunen, M. Binder, I. W. Dawes, and B. Hamilton.** 1997. The *Saccharomyces cerevisiae* peroxisomal 2,4-dienoyl-CoA reductase is encoded by the oleate-inducible gene *SPS19*. J. Biol. Chem. **272:**22140–22147.
25. **Hettema, E. H., C. W. T. van Roermund, B. Distel, C. Rodrigues-Pousada, R. J. A. Wanders, and H. F. Tabak.** 1996. The ABC transporter proteins Pat1 and Pat2 are required for import of long-chain fatty acids into peroxisomes of *Saccharomyces cerevisiae*. EMBO J. **15:**3813–3822.
26. **Hiltunen, J. K., B. Wenzel, A. Beyer, R. Erdmann, A. Fossa, and W. H. Kunau.** 1992. Peroxisomal multifunctional β-oxidation protein of *Saccharomyces cerevisiae*. Molecular analysis of the fox2 gene and gene product. J. Biol. Chem. **267:**6646–6653.
27. **Igual, J. C., E. Matallana, C. Gonzalez-Bosch, L. Franco, and J. E. Perez-Ortin.** 1991. A new glucose-repressible gene identified from the analysis of chromatin structure in deletion mutants of yeast SUC2 locus. Yeast **7:**379–389.
28. **Ishii, H., N. Fuicumori, S. Morie, and T. Suga.** 1980. Effects of fat content in the diet on the hepatic peroxisomes of the rat. Biochim. Biophys. Acta **617:**1–11.
29. **Issemann, I., and S. Green.** 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature **347:**645–650.
30. **Juge-Aubry, C., A. Pernin, T. Favez, A. G. Burger, W. Wahli, C. A. Meier, and B. Desvergne.** 1997. DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. J. Biol. Chem. **272:**25252–25259.
31. **Kal, A. J.** 1997. Transcriptional regulation of genes encoding peroxisomal proteins in *Saccharomyces cerevisiae*. Ph.D. thesis. Academic Medical Center of the University of Amsterdam, Amsterdam, The Netherlands.
32. **Karpichev, I. V., Y. Luo, R. C. Marians, and G. M. Small.** 1997. A complex containing two transcription factors regulates peroxisome proliferation and the coordinate induction of β-oxidation enzymes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:**69–80.
33. **Kliewer, S. A., S. S. Sundseth, S. A. Jones, P. J. Brown, G. B. Wisely, C. S. Koble, P. Devchand, W. Wahli, T. M. Willson, J. M. Lenhard, and J. M. Lehmann.** 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. Proc. Natl. Acad. Sci. USA **94:**4318–4323.
34. **Knoll, L. J., D. R. Johnson, and J. I. Gordon.** 1994. Biochemical studies of three *Saccharomyces cerevisiae* acyl-CoA synthetases, Faa1p, Faa2p, and Faa3p. J. Biol. Chem. **269:**16348–16356.
35. **Krey, G., O. Braissant, F. L'Horset, E. Kalkhoven, M. Perroud, M. G. Parker, and W. Wahli.** 1997. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol. Endocrinol. **11:**779–791.
36. **Lewin, A. S., V. Hines, and G. M. Small.** 1990. Citrate synthase encoded by the *CIT2* gene of *Saccharomyces cerevisiae* is peroxisomal. Mol. Cell. Biol. **10:**1399–1405.
37. **Liao, X., and R. A. Butow.** 1993. *RTG1* and *RTG2*: two yeast genes required for a novel path of communication from mitochondria to the nucleus. Cell **72:**61–71.
38. **Liao, X., W. C. Small, P. A. Srere, and R. A. Butow.** 1991. Intramitochondrial functions regulate nonmitochondrial citrate synthase (*CIT2*) expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**38–46.
39. **Luo, Y., I. V. Karpichev, R. A. Kohanski, and G. M. Small.** 1996. Purification, identification and properties of a *Saccharomyces cerevisiae* oleate-activated upstream activating sequence-binding protein that is involved in the activation of *POX1*. J. Biol. Chem. **271:**12068–12075.
40. **Marshall, P. A., Y. I. Krimkevich, R. H. Lark, J. M. Dyer, M. Veenhuis, and J. M. Goodman.** 1995. Pmp27 promotes peroxisomal proliferation. J. Cell Biol. **129:**345–355.
41. **McAlister-Henn, L., J. S. Steffan, K. I. Minard, and S. L. Anderson.** 1995. Expression and function of a mislocalized form of peroxisomal malate dehydrogenase (MDH3) in yeast. J. Biol. Chem. **270:**21220–21225.
42. **Melcher, K., M. Rose, G. H. Braus, and K. D. Entian.** 1995. Molecular analysis of the yeast *SER1* gene encoding 3-phosphoserine aminotransferase: regulation by general control and serine repression. Curr. Genet. **27:**501–508.
43. **Reddy, J. K., and T. P. Krishnakantha.** 1975. Hepatic peroxisome proliferation: induction by two novel compounds structurally unrelated to clofibrate. Science **190:**787–789.
44. **Rodriguez, J. C., G. Gil-Gomez, F. G. Hegardt, and D. Haro.** 1994. Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J. Biol. Chem. **269:**18767–18772.
45. **Rosenkrantz, M., T. Alam, K.-S. Kim, B. J. Clark, P. A. Srere, and L. P. Guarente.** 1986. Mitochondrial and nonmitochondrial citrate synthases in *Saccharomyces cerevisiae* are encoded by distinct homologous genes. Mol. Cell. Biol. **6:**4509–4515.
46. **Rottensteiner, H., A. J. Kal, M. Filpits, M. Binder, B. Hamilton, H. F. Tabak, and H. Ruis.** 1996. Pip2p: a transcriptional regulator of peroxisome proliferation in the yeast *Saccharomyces cerevisiae*. EMBO J. **15:**2924–2934.
47. **Rottensteiner, H., A. J. Kal, B. Hamilton, and H. F. Tabak.** 1997. A heterodimer of the Zn_2Cys_6 transcription factors Pip2p and Oaf1p controls induction of genes encoding peroxisomal proteins in *Saccharomyces cerevisiae*. Eur. J. Biochem. **247:**776–783.
48. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
49. **Schmalix, W., and W. Bandlow.** 1993. The ethanol-inducible *YAT1* gene from yeast encodes a presumptive mitochondrial outer carnitine acetyltransferase. J. Biol. Chem. **268:**27428–27430.
50. **Schoonjans, K., B. Staels, and J. Auwerx.** 1996. The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. Biochim. Biophys. Acta **1302:**93–109.
51. **Shani, N., P. A. Watkins, and D. Valle.** 1995. *PXA1*, a possible *Saccharomyces cerevisiae* ortholog of the human adrenoleukodystrophy gene. Proc. Natl. Acad. Sci. USA **92:**6012–6016.
52. **Stachelek, C., J. Stachelek, J. Swan, D. Botstein, and W. Konigsberg.** 1986. Identification, cloning and sequence determination of the genes specifying hexokinase A and B from yeast. Nucleic Acids Res. **14:**945–963.
53. **Stearns, T., M. A. Hoyt, and D. Botstein.** 1990. Yeast mutants sensitive to antimicrobial drugs define three genes that affect microtubule function. Genetics **124:**251–262.
54. **Su, S. S. Y., and A. P. Mitchell.** 1993. Molecular characterization of the yeast meiotic regulatory gene *RIM1*. Nucleic Acids Res. **21:**3789–3797.
55. **Suissa, M., K. Suda, and G. Schatz.** 1984. Isolation of the nuclear yeast genes for citrate synthase and fifteen other yeast mitochondrial proteins by a new screening method. EMBO J. **3:**1773–1781.
56. **Swartzman, E. E., M. N. Viswanathan, and J. Thorner.** 1996. The *PAL1* gene product is a peroxisomal ATP-cassette transporter in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. **132:**549–563.
57. **Tugwood, J. D., I. Issemann, R. G. Anderson, K. R. Bundell, W. L. McPheat, and S. Green.** 1992. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5′ flanking sequence of the rat acyl CoA oxidase gene. EMBO J. **11:**433–439.
58. **Van Der Leij, I., M. Franse, Y. Elgersma, B. Distel, and H. F. Tabak.** 1993. PAS10 is a tetratricopeptide-repeat protein that is essential for the import of most matrix proteins into peroxisomes of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90:**11782–11786.

59. **Van Roermund, C., E. Hettema, A. Kal, M. Van den Berg, H. Tabak, and R. Wanders.** 1998. Peroxisomal β-oxidation of polyunsaturated fatty acids in *Saccharomyces cerevisiae*: isocitrate dehydrogenase provides NADPH for reduction of double bonds at even positions. EMBO J. **17:**677–687.
60. **Veenhuis, M., M. Mateblowski, W. H. Kunau, and W. Harder.** 1987. Proliferation of microbodies in *Saccharomyces cerevisiae*. Yeast **3:**77–84.
61. **Wang, T., Y. Luo, and G. M. Small.** 1994. The *POX1* gene encoding peroxisomal acyl-CoA oxidase in *Saccharomyces cerevisiae* is under the control of multiple regulatory elements. J. Biol. Chem. **269:**24480–24485.
62. **Welch, J., S. Fogel, C. Buchman, and M. Karin.** 1989. The *CUP2* gene product regulates the expression of the *CUP1* gene, coding for yeast metallothionein. EMBO J. **8:**255–260.