Purification, Identification, and Properties of a Saccharomyces cerevisiae Oleate-activated Upstream Activating Sequence-binding Protein That Is Involved in the Activation of POX1*

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Peroxisomes have a central function in lipid metabolism, and it is well established that these organelles are inducible by many compounds including fatty acids. Peroxisomes are the sole site for the β-oxidation of fatty acids in yeast. The first and rate-limiting enzyme of this cycle is fatty acyl-CoA oxidase. The gene encoding this enzyme in Saccharomyces cerevisiae (POX1) undergoes a complex regulation that is dependent on the growth environment. When this yeast is grown in medium containing oleic acid as the main carbon source, peroxisomes are induced and POX1 expression is activated. When cells are grown in the presence of glucose, the expression of POX1 mRNA is repressed, whereas growth on a carbon source such as glycerol or raffinose causes derepression. This rigorous regulation is brought about by the complex interactions between trans-acting factors and cis-elements in the POX1 promoter. Previously, we characterized regulatory elements in the promoter region of POX1 that are involved in the repression and activation of this gene (Wang, T., Luo, Y., and Small, G. M. (1994) J. Biol. Chem. 269, 24480–24485). In this study we have purified and identified an oleate-activated transcription factor (Oaf1p) that binds to the activating sequence (UAS1) in the POX1 gene. The protein has a predicted molecular mass of approximately 118 kDa.

Peroxisomal β-oxidation is an important pathway in mammalian metabolism for catabolizing long and very long chain fatty acids. In many organisms, including yeasts, peroxisomal β-oxidation is the sole mechanism for the breakdown of fatty acids (1). The enzymes involved in this pathway are regulated according to the growth environment. Expression of genes encoding peroxisomal proteins in the yeast Saccharomyces cerevisiae is repressed when the yeast cells are grown in the presence of glucose, derepressed during growth on a nonfermentable carbon source, and activated when a fatty acid such as oleate is supplied for growth (2). This control is achieved through stringent transcriptional regulation of the genes encoding these proteins (3–8).

Over the past several years we have focused our attentions toward understanding the mechanisms that regulate genes encoding peroxisomal β-oxidation enzymes in S. cerevisiae. In order to address this question, we have concentrated on the regulation of POX1, the gene encoding acyl-CoA oxidase, the rate-limiting enzyme of this cycle. Previously we characterized two upstream repression sequences (URS1 and URS2)* and one upstream activating sequence (UAS1) in the promoter region of POX1 (7, 9). We demonstrated that a protein or protein complex binds to UAS1 in an oleate-dependent fashion, and this brings about the activation of POX1. A similar UAS sequence (termed oleate response element) was identified in the upstream regions of genes encoding some of the other peroxisomal proteins (3, 4, 10).

Several factors have been shown to be involved in the glucose repression of thiolase, the last enzyme in the peroxisomal β-oxidation cycle, which in S. cerevisiae is encoded by the FOX3 gene. This gene also undergoes regulation when the yeast is grown in glucose or oleate medium. The gene products of ADR1, SNF1, and SNF4 are all known regulators of glucose-repressible genes and have been shown to be positive regulators of FOX3 expression (5). However, mutations in the ADR1 or SNF4 genes appear to have little or no effect on the expression of POX1 (11), suggesting that different and/or additional factors are involved in the regulation of this gene.

Having identified some of the DNA elements that serve as binding sites for specific transcription factors involved in POX1 regulation, we have now turned our attentions to characterizing the biochemical properties of these trans-acting proteins. In order to achieve this goal it is necessary to purify and characterize these proteins. Here we describe the purification and molecular identification of a UAS1-binding oleate-activated transcription factor (Oaf1p). We demonstrate that the amount of Oaf1p that binds to UAS1 progressively increases during growth on oleate. Thus, oleic acid is required, either for the induction of Oaf1p itself or for its activation. We have utilized photo-affinity cross-linking to confirm the molecular weight of this protein. Furthermore, we have identified the gene (OAF1) encoding this transcription factor and have demonstrated that POX1 is not induced by oleate in a yeast strain that carries an OAF1 disruption.

MATERIALS AND METHODS

Yeast Strains and Culture Conditions—The S. cerevisiae strains 3A (ura3, trpl, arg4, ctt1) (12), BWG1–7a (lei2 ura3 ade1 his4) (13), and W3033A (lei2, ura3, trpl, ade2, his3) (14) were used for these experiments. Yeast were grown in YPD medium (2% yeast extract, 1% peptone, 2% glucose) and were induced in YPGO (1% yeast extract, 2% peptone, 3% glycerol, 0.1% oleic acid, 0.2% Tween 40). YPG medium contains 1% yeast extract, 2% peptone, and 3% glycerol. YNO was prepared according to Erdmann et al. (15). Oaf1p-DNA Binding as a Function of Growth in Oleate Medium—For...
the time course study ods (BWG3-7A) were grown for 8 h in YPD medium and were then shifted to YPGO medium. Samples were taken at each of the following time points: 0, 2, 4, 6, 8, 12, 14, and 18 h. Band shift experiments were carried out with labeled 80-mer containing the whole UAS1 or with a 184-mer containing URS2 and UAS1 (see Ref. 9 for details of DNA sequences). The intensity of the shifted band was quantified using Phospholmager software (Molecular Dynamics).

RNA Purification and Northern Analysis—Total yeast RNA was isolated using a slight modification of the guanidine thiocyanate procedure as described (16). The RNA was resolved in a formaldehyde-agarose gel and then transferred to Genescreen Plus nylon membrane (NEN Research products). The membranes were prehybridized for 1 h and then hybridized overnight in the presence of (u-32P)dATP-labeled probes (106 cpm/ml) at 65 °C using standard conditions (17). After extensive washing, the filters were exposed to a Phosphorlmager and scanned, and the intensities of the corresponding bands were determined using Molecular Dynamics Phospholmager software (Imagequant).

Preparation of a UAS1-Oligonucleotide Affinity Column—The oligonucleotides YL1 and YL2 (see Table I), which contain the upstream palindrome of the POX1 UAS1 (9), were annealed, self-ligated, and coupled to cyanogen bromide-activated Sepharose 4B according to the method described (18).

Purification of the Oleate-activated DNA-binding Protein—Oleate-induced yeast cells (strain 3A) (60 g) were harvested and resuspended in 50 ml of a lysis buffer (20 ml Tris-HCl, pH 8.0, 0.4 M NaCl, 10 mM MgCl2, 10 m M EDTA, 10% glycerol, 1 ml dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 1 ml protease inhibitor mixture containing 10 μg/ml leupeptin and antipain and 5 μg/ml chymotrysin and pepstatin). All further operations were performed at 4 °C. An amount of glass beads (0.5-mm diameter) equivalent to half of the cell suspension volume was added, and the cells were disrupted by vortexing for 20 min. Cell debris was removed by centrifugation (100,000 × g for 1.5 h). A saturated solution of ammonium sulfate was added to the supernatant to give a concentration of 25%. Following a 10–30 min incubation, the precipitated proteins were recovered by centrifugation and resuspended in buffer A (20 mM Hepes-NaOH, pH 7.9, 10% glycerol, 1 ml dithiothreitol, 1 ml dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride).

The cell extract was then passed through a cation exchange SP-Sepharose column (Pharmacia Biotech Inc.). Proteins were eluted using a linear KCl gradient (0–0.7 M) prepared in buffer A. Fractions containing the highest amount of OA1p (as judged by specific binding activity in a DNA band shift assay) were pooled and concentrated using a centrifugal filter device (Amicon). The concentrate was adjusted to 0.1 M KCl and then loaded onto a cetyl amine hydroxide polyacrylamide DNA cellulose column. Elution of proteins bound to the column was achieved using a KCl gradient, as above, and fractions with highest levels of OA1p activity were again pooled and concentrated. The concentrated sample was treated with DNase I (2 units) for 20 min at 37°C. SDS page-high performance liquid chromatography using a Beckman model 2100E gas phase sequencer with on-line PTH-derivative identification using minor modifications of the manufacturer’s programs.

Table I

| Name | Sequence |
|------|----------|
| YL1  | 5'-GATCTACGTTATAGTGTATAACTCGGAAG-3' |
| YL2  | 5'-GATCTCCGAGTTTTACACAACTTCAAGTA-3' |
| YL3  | 5'-CAGCCTCTTGCCTCACAGTAC-3' |
| YL4  | 5'-TTGAGCATCTTATATTATCGGTC-3' |
| YL5  | 5'-GGGTCGTACATTACGTATATTGGA-3' |
| YL6  | 5'-GATCTCATGTTATACAAATCTATGA-3' |
| YL7  | 5'-GATCTGATTATATACAAACTACCTCATCTATA-3' |

PCR Amplification of the Gene Encoding Oaf1p—In order to obtain a DNA fragment encoding the 118.2-kDa putative transcription factor, we designed oligonucleotides YL3 and YL4 (see Table I), which were complementary to the 5' and 3' ends of the DNA sequence obtained from the GenBank database. The oligonucleotides were used in a PCR amplification with genomic DNA isolated from S. cerevisiae strain W303A.

Disruption of the OAF1 Gene—To confirm that the cloned gene encodes the purified OA1-binding protein, we disrupted the genomic copy of this gene using a one-step gene disruption procedure (21). The DNA product obtained from the PCR amplification described above was cloned into pBluescript (Stratagene) and a 1.3-kb SpI fragment, containing part of the open reading frame, was excised. The ends of the plasmid were rendered blunt with T4 DNA polymerase, and a 1.8-kb Smal-Hindl fragment containing the HIS3 gene was inserted. The 3.8-kb OAF1::HIS3 insert was then excised and introduced into S. cerevisiae W303A, which had been previously transformed with a plasmid (pPOX1z) containing the lacz reporter gene under control of the POX1 promoter.

UV Cross-linking in a Gel Support—The complementary regions of oligonucleotides YL2 and YL5 (see Table I) containing the upstream palindrome of POX1 UAS1 were annealed, and the noncomplementary, overhanging regions were filled in with [32P]dCTP using Klenow fragment. The DNA fragment containing the 118.2-kDa putative transcription factor, was then dissected from the gel and treated with DNase I to produce a DNA fragment containing the putative transcription factor DNA fragment. The mixture was separated in 5% nondenaturing acrylamide gel. For experiments using a mutated version of the upstream palindrome of UAS1 as nonlabeled competitor DNA, oligonucleotides YL6 and YL7 (Table I) were annealed. Following electrophoresis, the gel was subjected to UV radiation (3000 uw/cm2) for 5 min at 4 °C and was then exposed to film for 1 h. The shift in the gel was visualized using a Bio-Imager (using a specific protein-DNA complex was located by lining up the gel with the film, and the gel slice in this region was excised. The gel slice was then incubated with 40 μl of SDS sample buffer (120 mM Tris, pH 6.8, 4% SDS, 20% sucrose, 0.002% bromphenol blue, 20 mM dithiothreitol) for 15 min at 68 °C, loaded into the well of an 8% SDS-polyacrylamide gel, and electrophoresed for 2 h at 70 volts. The gel was subsequently dried and subjected to autoradiography.

UV Cross-linking in Solution—A binding mixture that contained 1 μl of a competitor DNA, poly(dI-dC), was prepared. This solution was directly exposed to UV radiation (3000 uw/cm2) for 7 min. The sample was treated with DNPase I (2 units) for 20 min at 37 °C. SDS sample buffer was then added to the DNase-treated sample, and the matrix was separated in 5% nondenaturing acrylamide gel. For experiments using a mutated version of the upstream palindrome of UAS1 as nonlabeled competitor DNA, oligonucleotides YL6 and YL7 (Table I) were annealed. Following electrophoresis, the gel was subjected to UV radiation (3000 uw/cm2) for 5 min at 4 °C and was then exposed to film for 1 h. The shifted band was visualized using a Bio-Imager (using a specific protein-DNA complex was located by lining up the gel with the film, and the gel slice in this region was excised. The gel slice was then incubated with 40 μl of SDS sample buffer (120 mM Tris, pH 6.8, 4% SDS, 20% sucrose, 0.002% bromphenol blue, 20 mM dithiothreitol) for 15 min at 68 °C, loaded into the well of an 8% SDS-polyacrylamide gel, and electrophoresed for 2 h at 70 volts. The gel was subsequently dried and subjected to autoradiography.
Oaf1p Binding as a Function of Growth in Oleate Medium—We wished to determine the kinetics of Oaf1p activation and whether Oaf1p-UAS1 binding activity could be detected immediately when cells were shifted from glucose to oleate medium. To this end, we carried out a time course study to determine the level of UAS1 binding of Oaf1p. We carried out a DNA band shift experiment with cell extracts from each time point. These experiments were performed either with a 184-mer, which contains both URS2 and UAS1 (Fig. 1a), or an 80-mer, which contains UAS1 alone (Fig. 1b), as DNA probes. This pattern was used in the assay.

Other Methods—DNA band shift, protein, and β-galactosidase assays were performed as described previously (9).

RESULTS

Oaf1p Binding as a Function of Growth in Oleate Medium—We wished to determine if the kinetics of Oaf1p induction correlated with the binding of Oaf1p to UAS1. For this purpose we used strain Pp13570, which contains the lacZ gene under the control of the POX1 promoter (9), for the time course study shown in Fig. 1. Thus, we could measure the expression of POX1 and the activity of β-galactosidase driven by the POX1 promoter. Total RNA was isolated at various time points, and the abundance of POX1 message was analyzed by Northern blot using a DNA probe consisting of the coding region of POX1. The relative abundance of POX1 message was quantified by normalizing the mRNA levels to those of phosphoglycerol kinase (PGK1) for each time point. This analysis revealed an intriguing pattern of expression. The POX1 transcript was not detectable in glucose-grown cells but was rapidly induced following the shift to oleate medium (Fig. 2a). The expression increased rapidly during the first 2–4 h following the switch to oleate medium, after which the level slowly declined up until the 12-h time point. However, at 14 and 18 h the level of POX1 message was increased (Fig. 2, a and b). This fluctuation was not due to unequal loading of the gel as determined by ethidium bromide-staining of the agarose gel (data not shown), and the pattern in POX1 expression was the same whether compared with that of PGK1 (Fig. 2) or with actin (ACT1, data not shown). As a second, independent method to quantify induction of POX1, we measured the POX1 promoter-dependent expression of β-galactosidase in samples of cell extracts taken at each time point. The activity followed a similar pattern to that of POX1 expression in that the β-galactosidase activity reached a maximum value at 6–8 h of induction (Fig. 2b). The activity decreased at the 12-h time point; however, by 18 h the activity increased slightly. Whether these fluctuations are caused by protein and/or mRNA degra-
dation or due to transcriptional control is unclear.

Purification of the UAS1-binding Protein—POX1 expression is activated in yeast cells grown in YPGO medium. By means of DNA band shift analysis, we have shown that this induction corresponds with the binding of a protein or proteins to a specific sequence, designated UAS1, in the POX1 promoter. As a first step toward understanding the mechanisms by which activation of this POX1 UAS1 element mediates oleate-specific induction of this gene, we purified a protein that binds to this regulatory element. Details of the purification protocol are given under "Materials and Methods." As one of the purification steps, we carried out double-stranded calf thymus DNA cellulose chromatography. The absorbance and Oaf1p activity profiles of fractions eluted from this DNA cellulose column are shown in Fig. 3a. We estimate that we recovered approximately 30% of the UAS1-binding protein during this step of the purification (Table II). Oaf1p DNA binding activity was followed by performing a DNA band shift assay with alternate fractions, and the highest activity was recovered in fractions 6–10 (Fig. 3b, lanes 5–7).

Fractions 6–10 from the DNA cellulose column were concentrated and loaded onto a UAS1 affinity column. The bulk of the nonspecific proteins did not bind to this column, and Oaf1p DNA binding activity was eluted at a KCl concentration between 0.4–0.6 M in fractions 4–10 (Fig. 4a, lanes 6–9). SDS-polyacrylamide gel electrophoresis of the fractions eluted from the affinity column revealed by silver staining protein bands that appear to consist of a doublet, with an apparent molecular mass of approximately 110–120 kDa (Fig. 4b, lanes 7–9). We were unable to detect Oaf1p in fraction 4 by silver staining, even though we found DNA binding activity in this fraction (compare Fig. 4, a and b). The reason for this is not clear; however, much less protein sample was used in the DNA band shift assay than was loaded onto the SDS gel (1 μl compared with 20 μl). Thus, it is possible that the discrepancy is due to experimental error if a volume slightly greater than 1 μl was inadvertently added to the band shift assay. Nevertheless, the
strongest silver-stained band was recovered in fraction 8, which was also the fraction with the greatest DNA binding activity. The protein concentration of these fractions was too low to measure by standard techniques, thus we are unable to give accurate and specific recoveries for this step of the purification. However, by comparing the intensity of the bands on the silver-stained gel with that of a known concentration of protein from the molecular weight standards and from the band shift assays, we estimate that approximately 7% of the Oaf1p from the original lysate was recovered following affinity chromatography. This gave approximately an 18,000-fold purification (Table II).

Verification of the Specificity of Oaf1p Binding—Throughout the different stages of chromatography that we used to purify the UAS1-binding protein, we carried out DNA band shift experiments to identify those fractions that contained activity. In order to confirm that the band shift detected was specific for binding of Oaf1p to UAS1, we carried out the band shift assay with fractions eluted from the SP column, in the presence and the absence of various competitive DNA (100-fold excess). A uniformly [32P]dCTP-labeled BrdUrd-UAS1 probe was incubated with fractions eluted from the SP column containing partially purified Oaf1p in the presence or the absence of competitor DNA. BrdUrd was substituted for thymidine in these experiments so that the probe could be used for subsequent cross-linking experiments. DNA-protein complexes were identified by DNA band shift assays. An 80-nucleotide DNA fragment containing UAS1 (80-mer) or annealed oligonucleotides corresponding to the upstream palindrome of UAS1 successfully competed for the binding of labeled UAS1 (Fig. 5a, lanes 2 and 3). The CGG sequence contained in each repeat of UAS1 is also conserved in the UAS1/oleate response element sequences present in other genes encoding peroxisomal β-oxidation enzymes in S. cerevisiae. We designed oligonucleotides YL6 and YL7, corresponding to the upstream palindrome of UAS1, in which each CGG sequence is mutated to TAA (Table I). These oligonucleotides, when annealed and self-ligated, do not specifically bind to a protein in DNA band shift assays carried out with extracts from oleate-grown cells (data not shown). We used these oligonucleotides (mut-UAS1) as a competitor for the band shift obtained with labeled UAS1, and no competition was observed (Fig. 5a, lane 4).

Photoaffinity Cross-linking of Oaf1p to UAS1—In order to identify the specific protein(s) that contact the DNA, the gel shown in Fig. 5a (lanes 1–4) was exposed to ultraviolet light, and Oaf1p-UAS1 complexes were excised and electrophoresed into an 8% SDS-polyacrylamide gel. Assembled protein-DNA complexes are cross-linked following exposure to UV radiation; therefore this procedure can be used to identify proteins that bind to a specific DNA sequence. We used this method to identify Oaf1p and confirm the molecular mass of this protein or protein complex. Radiolabeled DNA-protein complexes were identified by autoradiography (Fig. 5b, lanes 1–4). Two bands were visible; one had a molecular mass of approximately 136 kDa. Adjusting for the presence of associated DNA, we estimate that the protein(s) in this DNA-protein complex has a molecular mass of approximately 120 kDa. The second band had a mass of greater than 200 kDa (Fig. 5b, lanes 1 and 4). Both of these bands were specific for UAS1 because they were not present in lanes 2 and 3, in which excess unlabeled UAS1 had competed for the binding of labeled UAS1. A similar result was obtained when the cross-linking experiment was performed with a fraction containing Oaf1p, eluted from the affinity column (Fig. 5b, lanes 5–7). Cross-linking of nonspecific DNA-protein complexes did not give rise to either band (data not shown); thus, the high molecular weight band seen in Fig. 5b is not due to nonspecific aggregation of complexes during electrophoresis but rather could represent a dimerized form of the Oaf1p-UAS1 complex.

In order to verify the results obtained above, we carried out a similar cross-linking experiment in which the mixture of labeled DNA and a partially purified fraction containing Oaf1p activity was exposed to UV radiation in solution. This reaction was carried out in the presence or the absence of competitor DNA, and the mixture was then treated with DNase I to degrade any DNA that was not protected by Oaf1p. Following separation of the products by SDS-polyacrylamide gel electrophoresis, several cross-linked bands appeared (Fig. 5b, lanes 9–11). However, only the protein-DNA complex at approximately 136 kDa was specifically competed out with oligonucleotides containing UAS1 (lane 10) but not with mutated UAS1 (lane 11). Thus, the DNase I treatment did not affect the mobility of this protein-DNA complex, suggesting that most of this DNA sequence is protected by bound Oaf1p.

Taken together, these results strongly indicate that the 120-kDa protein purified by our affinity chromatography strategy is Oaf1p. We cannot exclude the possibility that Oaf1p consists of
two different proteins or protein subunits that have similar molecular masses rather than of a single protein, which gives rise to a dimerized form of approximately 200 kDa. Microsequence Analysis of Oaf1p—Oaf1p was shown to be identical to a hypothetical protein from yeast, described in the GenBank sequence data base as a "putative 118.2-kDa transcriptional regulatory protein in ACS-1-PTA-1 intergenic region" (GenBank accession number P39720). However, prior to this report, the protein had not been purified nor functionally characterized but was known only by the deduced amino acid sequence and by the presence of specific motifs; it has both transmembrane and DNA-binding motifs and is presumably localized to the nucleus. Direct amino acid sequence analysis of tryptic peptides from purified Oaf1p were each used to search the GenBank data bases, and both showed 100% identity to amino acid sequences in the 118.2-kDa hypothetical protein, as shown in Table III. In addition, these peptides were consistent with tryptic cleavage carboxyl-terminal to lysyl residues. Finally, the amino acid sequence of a pentapeptide was also determined and could be found in the 118.2-kDa sequence (DMFYW, residues 118–122; cleavage after Lys117). While providing further confirmation, this peptide was considered too short for a reliable independent search of the data base. The sequence of Oaf1p from amino acids 66 to 97 shows a high level of structural homology to the DNA-binding "fingers" found in many regulatory proteins. Fig. 6 shows a comparison of this region of Oaf1p with two other yeast regulatory proteins, the products of CYP1 (23) and GAL4 (24).

Disruption of the Chromosomal Copy of the Gene Encoding Oaf1p—By PCR amplification using oligonucleotides YL3 and YL4 (Table I), which are homologous to the 5' and 3' ends of the gene encoded by P39720, we obtained a DNA fragment of the size predicted (3.2 kb) to encode OAF1. We confirmed that it was the expected DNA fragment by restriction enzyme analysis and by sequencing 100 nucleotides at both the 3' and 5' ends.

A 1.3-kb Sphl fragment of the open reading frame of OAF1 was replaced with the yeast HIS3 gene as described under "Materials and Methods." The successful disruption of OAF1 was confirmed by using genomic DNA from the parental and disrupted strain in a PCR assay with oligonucleotides YL3 and YL4, previously used to clone the gene. The DNA product from the transformants was approximately 0.5 kb larger than that obtained from the parental strain, confirming that the OAF1::HIS3 fragment was integrated into the genome.

The effect of disrupting OAF1 was tested in four ways. Firstly we tested whether the disrupted strain could grow on plates containing oleic acid but no glycerol (YN0 medium). Yeast strains that lack functional β-oxidation enzymes are not able to grow on YNO plates (12, 15). Our parental strain grew, although slowly, on this medium, whereas the disrupted strain was unable to grow (Fig. 7a), suggesting that the β-oxidation pathway was not induced in the presence of oleate in this strain. We then prepared extracts from both parental and disrupted strains grown in YPGO medium and used the extracts in a DNA band shift assay with labeled UAS1. No band shift was seen with extracts from the disrupted strain grown in the presence of oleate (Fig. 7b, lane 4), whereas the expected shift was present with extracts from the parental strain (Fig. 7b, lane 2). The lower, lower band seen in this gel is nonspecific because it is not competed out by excess probe (data not shown, but see Ref. 9).

As further confirmation that we had disrupted the gene encoding the transcription factor responsible for the oleate-induction of POX1, we transformed the disrupted strain with a centromeric plasmid containing the OAF1 gene. We then performed a Northern blot on the parental, disrupted, and rescued strains grown in glycerol or oleate medium and measured the level of POX1 mRNA (Fig. 8a). The levels of PGK1 mRNA were also measured as a control for RNA loading (Fig. 8b). The amount of RNA loaded in each lane is somewhat uneven; however it is clear that POX1 expression is not induced in the OAF1 disrupted strain (Fig. 8a, lanes 3 and 4). According to the level of PGK1 expression (measured by PhosphorImager analysis), the amount of RNA loaded in lane 6 was 1.5-fold greater than that loaded in lane 5. However, POX1 expression was 7.5-fold greater in the oleate-induced cells (lane 6) compared with cells grown in glycerol (lane 5). Thus, allowing for unequal loading, POX1 is induced approximately 5-fold in the strain rescued with the OAF1 gene.

Finally, the parental and disrupted strains also carried an
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Fig. 8. POX1 expression in parental (P), OAF1-disrupted (K-O), and OAF1-rescued (R) strains grown in YPG (G) or YPGO (O) medium. a and b, Northern blot analysis of POX1 and PGK mRNA expression, respectively. c, β-galactosidase activities measured in extracts prepared from the same cells used for RNA extraction in a and b.

integrated copy of the lacZ gene under control of the POX1 promoter. Therefore, in addition to the Northern analysis, we also measured the β-galactosidase activity in each strain (Fig. 8c). The results confirmed that the POX1 promoter was not activated by oleate in the OAF1-disrupted strain but was activated by oleate in both the parental and rescued strains. Together these results confirm that we have cloned the gene encoding the UAS1-binding protein and that disruption of this gene prevents the encoded protein from binding to UAS1 and thus activating POX1.

DISCUSSION

In an earlier report we demonstrated that a protein or proteins bind to a UAS1 element in the POX1 promoter in an oleate-activated fashion and that this causes transcriptional activation of the POX1 gene (9). Here we describe the use of a standard chromatography strategy for the purification of the binding protein using as a final step a DNA affinity column containing the UAS1 binding site (18). The oleate-specific binding protein(s) (Oaf1p) appears as a doublet or triplet on a silver-stained gel and has an apparent molecular mass of approximately 120 kDa. Photoaffinity cross-linking data confirmed the size and specificity of this DNA-binding protein.

We find that the amount of Oaf1p binding to UAS1 increases following a shift of cells from glucose to oleate medium, suggesting that this transcription factor is activated and/or induced continuously in the presence of oleate. The protein that binds to URS2 appears to be present in cell extracts regardless of the growth conditions tested. However, in this in vitro assay, it is likely that the two proteins are bound to different molecules of DNA because there is no evidence of a “super-shift” in the DNA band shift assay, and this would be expected if both proteins were bound to the same DNA molecule.

Oaf1p was recently identified as YAL051W, an open reading frame on the left arm of chromosome I (25). The gene product is a transcription factor that binds to the URS elements of the POX1 promoter and this prevents transcription of the gene. When the cells are shifted to an oleate medium, Oaf1p may become modified (perhaps phosphorylated) and bind to the UAS1. This would over-ride the repressing activity of the URS protein(s) and cause activation of the gene.

The model outlined above is a much simplified version of the mechanisms that we believe operate to control repression, derepression, and activation of POX1. It is likely that expression of genes encoding peroxisomal proteins is coordinately mediated by a specific subset of proteins. It was recently shown that two genes, RTG1 and RTG2, are required for efficient growth of S. cerevisiae on oleate medium (30). These genes are involved in the basal and induced expression of the CIT2 gene (31). CIT2 encodes peroxisomal citrate synthase, a glyoxylate cycle enzyme (32, 33). The RTG1 product is a transcription factor that binds to UAS5 in the CIT2 promoter (31). This protein does not bind to the oleate response element of the POX3 gene, and it was suggested that the RTG genes may act at an early step in the signal transduction pathway leading to peroxisomal induction (30).

We are conducting experiments in our laboratory to isolate and characterize the factors involved in the pathway that regulates POX1. Experiments are also underway to further characterize Oaf1p. We will use the strain in which Oaf1p is disrupted in order to gain important information regarding the role of Oaf1p in the regulation of other genes encoding peroxisomal enzymes.

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