Saccharomyces cerevisiae Adr1p Governs Fatty Acid β-Oxidation and Peroxisome Proliferation by Regulating POX1 and PEX11*

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Saccharomyces cerevisiae Adr1p is essential for fatty acid degradation and peroxisome proliferation. Here, the role of Adr1p was examined with respect to the transcriptional regulation of the Pip2p-Oaf1p dependent genes POX1 and PEX11. POX1 encodes the rate-limiting enzyme of peroxisomal β-oxidation, acyl-CoA oxidase. The POX1 promoter was shown to contain a canonical Adr1p element (UAS1), within which the oleate response element (ORE) was nested. PEX11 codes for a peroxin that is critical for normal peroxisome proliferation, and its promoter was shown similarly to contain a UAS1-like element overlapping the ORE. Northern analysis demonstrated that transcriptional up-regulation of both POX1 and PEX11 was abolished in adr1Δ mutant cells, and immunoblotting confirmed that the abundance of their gene products was dramatically reduced. Studies of an overlapping ORE/UAS1 arrangement in the CTA1 promoter revealed synergy between these elements. We conclude that overlapping ORE and UAS1 elements in conjunction with their binding factors Pip2p-Oaf1p and Adr1p coordinate the carbon flux through β-oxidation with peroxisome proliferation.

Defective peroxisomal β-oxidation has serious implications to human patients, with death occurring often within the first year of life (1, 2). The largest group of peroxisomal diseases consists of aberrations in β-oxidation caused by a single defective enzyme (1). Defects resulting in a drop in the carbon flux through β-oxidation additionally impede the scheduled expansion of the peroxisomal compartment both in human cells (3) and in those of the yeast Saccharomyces cerevisiae (4). This study is concerned with the molecular basis for the defects in β-oxidation and peroxisome proliferation in yeast cells devoid of Adr1p (5).

In S. cerevisiae, peroxisomes represent the exclusive site for fatty acid β-oxidation (6). Supplying S. cerevisiae cells with oleic acid as a sole carbon source causes them to induce the transcription of genes involved in fatty acid β-oxidation by about 10-fold (7, 8) and also to increase dramatically the number and size of their peroxisomes (7). This dual response to oleic acid is mediated by the Pip2p-Oaf1p transcription factor, which binds to a promoter element termed ORE1 in target genes (8, 9). Cells devoid of this transcription factor fail to degrade oleic acid or expand their peroxisomal compartment (8, 9).

The dual response to oleic acid is also severely impaired in adr1Δ mutant cells, which similarly are unable to break down oleic acid or proliferate their peroxisomes (5, 10). The Adr1p transcription factor has been identified previously as a regulator of the alcohol dehydrogenase gene ADH2 (11). Adr1p binds to the consensus sequence C(T/C)CC(A/G)(A/T/G)N1–36 (T/A/C)(T/C)GG(A/G)G, termed UAS1 (12). Adr1p is also known to regulate the transcription of the gene for peroxisomal catalase A by binding directly to UAS1CTA1 (5); however, the influence of Adr1p on the transcription of genes encoding peroxisomal β-oxidation enzymes such as Fox2p and Pot1p/Fox3p was reported to be less pronounced and possibly indirect (13, 14). Interest in the role of Adr1p in peroxisome function was regained recently by the finding that Adr1p tightly regulates SPS19 encoding peroxisomal 2,4-dienoyl-CoA reductase by binding to UAS1SPS19 (15, 16). However, because neither CTA1 nor SPS19 are essential for growth on oleic acid, the precise role of Adr1p in regulating either the carbon flux through the β-oxidation spiral or peroxisome proliferation is still not understood fully.

With respect to the β-oxidation phenotype of adr1Δ mutant cells, a canonical UAS1 (CTCCGAG36TTGGGG) occurs at position −247 upstream of the ATG start site of POX1 (12), the product of which, acyl-CoA oxidase, represents the first and rate-limiting step of the β-oxidation spiral (17). Hence, if POX1 were to turn out to be regulated by Adr1p, it could explain why the adr1Δ mutant strain fails to degrade oleic acid. As for the reduced size and number of peroxisomes in the adr1Δ mutant (10), a possible clue for this might lie in the similarity in the situation to pip2Δ and oaf1Δ strains (8, 9). These latter two mutants have smaller and fewer peroxisomes because they fail to transcribe properly the Pip2p-Oaf1p dependent gene PEX11 (8). Pex11p is vital for proper proliferation of an otherwise readily discernible peroxisomal compartment (18, 19) and is also critical for Faa2p-dependent activation of medium-chain fatty acids prior to β-oxidation (4). According to the yeast data bases, the PEX11 promoter contains UAS1-like sequences; therefore, should the gene be demonstrated to depend on Adr1p for its transcription, this could shed additional light on the

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In the present study, the role of Adr1p was examined with respect to the regulation of POX1 and PEX11. We also studied the significance of the close proximity between OREs and POT1/FOX3 with respect to the regulation of mutant.

The construction of strains MF14, MF17, and MF22 or in the corresponding strains MF14 and MF14/H9004 derived from BJ1991/H9251 MAT oaf1/H9004 –adr1/H9004, the construction of strains MF14 and MF14/H9004 for producing recombinant Adr1p-LacZ from plasmid pQC-A229Z (20).

The terminal 3′-CCG tripeptide is 112 nucleotides 5′ of the ATG start site. B, the overlap between the analogous two elements in the SPS19 promoter. The ATG start site occurs 127 nucleotides 3′ of the sequence depicted. C, the POX1 promoter fragment containing UAS1POX1 and POX1 ORE. The terminal 3′ nucleotide is situated 245 nucleotides 5′ of the ATG start site. D, EMSA using POT1/FOX3 ORE or UAS1CTA1 Labeled DNA containing POT1/FOX3 ORE or UAS1CTA1 was mixed with soluble protein extracts from wild-type, adr1Δ, or pip2Δoaf1Δ yeast cells or E. coli cells producing recombinant Adr1p-LacZ as indicated, and free and bound DNA fragments were resolved on a 5% (w/v) polyacrylamide gel. A 25-fold excess of unlabeled double-stranded oligonucleotides was used for competition. The arrows with asterisks indicate unidentified complexes.

YEAST extract/2% (w/v) meat peptone medium containing 2% (w/v) n-glucose, shifted to yeast extract/peptone medium containing either 4% (w/v) d-glucose, 2.5% (v/v) ethanol, or 0.2% (w/v) oleic acid and 0.02% (w/v) Tween 80 (adjusted to pH 7.0 with NaOH), and aerated vigorously with shaking for 8 h in 4% (w/v) glucose medium (to an A600 < 1.0) or for 20 h in ethanol or oleic acid media.

Immunoblotting—Whole-cell extracts were prepared according to the published protocol (25). Monoclonal anti-yeast 3-β-phosphoglycerate kinase (Ppg1p) antibodies were obtained from Molecular Probes (Eugene, OR). Antibodies against Fox3p (26) or Pex11p (18) have been described previously. The antibody against Cta1p was a gift from the laboratory of Dr. H. F. Tabak, and that against Pox1p was a gift from the laboratory of Dr. W.-H. Kunau. Immunoreactive complexes were visualized using anti-rabbit or anti-mouse IgG-coupled horseradish peroxidase in combination with the ECL™ system from Amersham Pharmacia Biotech.

Enzyme Assays—β-Galactosidase activities were assayed in soluble protein extracts prepared by breaking cells with glass beads (27) and are expressed as nmol of O-nitrophenyl-β-D-galactopyranoside hydrolyzed/min·mg of protein. Catalase measurements were performed as described (28). Acyl-CoA oxidase activity was measured as the production of trans-2-decenoyl-CoA from the substrate n-decanyl-CoA (Sigma). The rate of trans-2-decenoyl-CoA generation was monitored spec-

FIG. 1. Adr1p is critical for Pox1p and Pex11p expression in cells grown on oleic acid medium. A, the effect of disrupting ADR1 on POX1 and PEX11 transcription. Logarithmic cultures of wild-type and mutant strains were inoculated in media supplemented with the indicated carbon sources and grown for 16 h. RNA extracted from these strains was immobilized on a filter that was probed with labeled DNA fragments containing the genes indicated. The strains used were MF14, MF14adr1Δ, MF14/pip2Δ, and MF14adr1Δ/pip2Δ. B, immunoblotting using soluble protein extracts from cells grown in the media indicated. The strains used were BJ1991 wild type, BJ1991adr1Δ, and BJ1991pip2Δoaf1Δ.

FIG. 2. Competition EMSA of POX1 promoter fragments showing interaction with Pip2p-Oaf1p and Adr1p. A, diagram of the PEX11 promoter section covering the UAS1-like element and the ORE. The terminal 3′-CCG tripeptide is 112 nucleotides 5′ of the ATG start site. B, the overlap between the analogous two elements in the SPS19 promoter. The ATG start site occurs 127 nucleotides 3′ of the sequence depicted. C, the POX1 promoter fragment containing UAS1POX1 and POX1 ORE. The terminal 3′ nucleotide is situated 245 nucleotides 5′ of the ATG start site. D, EMSA using POT1/FOX3 ORE or UAS1CTA1 Labeled DNA containing POT1/FOX3 ORE or UAS1CTA1 was mixed with soluble protein extracts from wild-type, adr1Δ, or pip2Δoaf1Δ yeast cells or E. coli cells producing recombinant Adr1p-LacZ as indicated, and free and bound DNA fragments were resolved on a 5% (w/v) polyacrylamide gel. A 25-fold excess of unlabeled double-stranded oligonucleotides was used for competition. The arrows with asterisks indicate unidentified complexes.

yeast extract/2% (w/v) meat peptone containing the indicated carbon sources and grown for a further 16 h (8). For β-galactosidase measurements, cells were induced in oleic acid medium as described (16). For immunoblotting, cells were induced in yeast extract/peptone medium containing 2% (w/v) n-glucose, shifted to yeast extract/peptone medium containing either 4% (w/v) d-glucose, 2.5% (v/v) ethanol, or 0.2% (w/v) oleic acid and 0.02% (w/v) Tween 80 (adjusted to pH 7.0 with NaOH), and aerated vigorously with shaking for 8 h in 4% (w/v) glucose medium (to an A600 < 1.0) or for 20 h in ethanol or oleic acid media.

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Expression of Pox1p and Pex11p Depends on Adr1p—The promoters of both POX1 and PEX11 contain potential UAS1s. However, unlike the canonical UAS1 in the POX1 promoter (12), the promoter of PEX11 contains two UAS1-like variants. One occurs at position −119 (TTCATCTCGGAG, nucleotides in bold diverge from the palindromic consensus) with the 3′ half-site situated within the PEX11 ORE. A second potential element in the PEX11 promoter, occurring as a tandem array of two half-sites (CTCCCATGAGCAGTCCCG), can be found relatively close to the ATG start site at position −42.

To determine whether Adr1p is important for transcribing POX1 or PEX11, a Northern blot was used to study transcription in cells grown under various carbon source conditions. RNA was extracted from wild-type and adr1Δ mutant cells as well as from control cells lacking either Pip2p or the specific activator of ORE-regulated genes (8), or both Pip2p and Adr1p. The results showed that the adr1Δ deletion caused a complete loss of induction of both POX1 and PEX11 in cells grown on oleic acid (Fig. 1A). This defect was similar to that caused by the pip2Δ deletion. The Northern blot results were validated using probes representing the genes POT1/FOX3 and CTA1, which are subordinate to varying extents to both Pip2p-Oaf1p and Adr1p (8, 9, 13, 14, 21, 35). ACT1 represented a control gene, the regulation of which is independent of either transcription factor (Fig. 1A). The data indicated that Adr1p was probably important for POX1 and PEX11 transcription.

To substantiate the apparent requirement for Adr1p to express Pox1p and Pex11p, immunoblotting was performed on soluble protein extracts from wild-type, adr1Δ, or pip2Δoaf1Δ cells. The results demonstrated that under oleic acid-medium conditions, Adr1p was essential for obtaining induced levels of Pox1p and Pex11p, i.e. for increasing the amount of protein beyond the levels already seen on the ethanol medium (Fig. 1B). This resembled the situation with cells lacking Pip2p-Oaf1p (Fig. 1B). In comparison, Cta1p and particularly Pot1p/Fox3p were less stringently regulated by Adr1p. Pkg1p served as an internal control for equal loading of protein (Fig. 1B). The dependence of Pox1p expression on Adr1p was underscored by assaying for acyl-CoA oxidase activity in soluble protein extracts from oleic acid-grown cells. The results showed that although oxidase activity measured in the wild-type extracts reached 0.17 ± 0.04 (mean ± S.D., n = 3) nmol × min⁻¹/mg of protein, oxidase activity in extracts obtained from adr1Δ, pip2Δoaf1Δ, or pox1Δ mutant cells was below the detection limit of 0.02 nmol × min⁻¹/mg of protein. Hence, the induced expression of Pox1p and Pex11p under oleic acid medium conditions strictly depended on Adr1p (in addition to Pip2p-Oaf1p), arguing in favor of a blockage in both β-oxidation and peroxisome proliferation in adr1Δ mutant cells.

UAS1POX1 Interacts with Adr1p—We then tested whether the canonical UAS1 on the POX1 promoter (36) in that its promoter contains a potential Adr1p element with two half-sites arranged as a direct repeat. In addition, the PEX11 promoter also has a potential UAS1/ORE overlap similar to that in SPS19 (16) (Fig. 2A and B). Although the issue of whether Adr1p directly regulates PEX11 remains open, the binding properties of these two classes of element arrangements, i.e. linear or ORE overlapping, have been investigated previously. On the other hand, the POX1 promoter contains a novel arrangement that consists of a potential UAS1POX1, which is nested the entire POX1 promoter (36) (Fig. 2C). To study the protein-binding properties of the two superimposed elements in POX1, an electrophoretic mobility shift assay (EMSA) was performed using competitor DNA fragments generated from annealed oligonucleotide pairs. The results demonstrated that the addition of soluble protein extracts from wild-type cells grown on oleic acid to labeled POT1/Fox3 ORE gave rise to a Pip2p-Oaf1p complex (8, 9) that was missing from the extract derived from similarly grown pip2Δoaf1Δ cells (Fig. 2D, lanes 2 and 3). The addition of excess POT1/Fox3 ORE (self-competitor DNA) caused this signal to disappear (lane 4). Similarly, excessive amounts of either POX1 ORE or UAS1POX1 were also able to compete for the Pip2p-Oaf1p complex (lanes 7 and 8). As anticipated, this Pip2p-Oaf1p complex could also be competed by adding excess SPS19 ORE but not UAS1SPS19 (lanes 5 and 6).

To resolve the two overlapping promoter elements with respect to their ability to bind Adr1p, a competition EMSA was...
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The effect of adr1Δ and pip2Δ deletions on the expression of reporter genes based on CYC1-lacZ or on native CTA1 under ethanol or oleic acid medium conditions

All measurements were performed in duplicate.

| Strains | Ethanol | Oleic acid | Relative level | Catalase activity |
|---------|---------|------------|----------------|-------------------|
|         | nmol min⁻¹/mg of protein | units/mg of protein |                  |                   |
| basal reporter (pMF6<sup>a</sup>) |         |            |                |                   |
| MF6 wild type | 10.1 | 3.9 | 1 | 91.0 | 526.3 |
| MF6adr1Δ | 27.8 | 4.3 | 10.3 | 49.1 |
| MF6pip2Δ | 13.4 | 2.2 | 107.6 | 52.7 |
| MF6adr1Δpip2Δ | 25.1 | 6.3 | 19.0 | 11.9 |
| both UAS<sub>1</sub>CTA1 and CTA1 ORE (pM14) |         |            |                |                   |
| MF14 wild type | 74.7 | 289.0 | 74.1 | 91.2 | 463.5 |
| MF1adr1Δ | 24.2 | 48.6 | 10.6 | 43.8 |
| MF14pip2Δ | 66.1 | 11.2 | 91.8 | 43.7 |
| MF1adr1Δpip2Δ | 27.5 | 2.8 | 17.1 | 4.4 |
| UAS<sub>1</sub>CTA1 only (pMF17) |         |            |                |                   |
| MF17 wild type | 41.6 | 66.6 | 17.1 | 78.5 | 248.3 |
| MF17adr1Δ | 2.2 | 1.5 | 11.4 | 29.6 |
| MF17pip2Δ | 40.0 | 15.8 | 81.6 | 55.3 |
| MF17adr1Δpip2Δ | 1.7 | 2.1 | 13.4 | 7.6 |
| CTA1 ORE only (pMF22) |         |            |                |                   |
| MF22 wild type | 13.6 | 50.7 | 13.0 | 75.0 | 307.5 |
| MF22adr1Δ | 14.2 | 19.4 | 15.7 | 66.7 |
| MF22pip2Δ | 19.9 | 1.8 | 68.1 | 66.7 |
| MF22adr1Δpip2Δ | 11.2 | 2.7 | 14.8 | 9.4 |

TABLE I

*Integrative plasmid pMF6 is loaded with a basal CYC1-lacZ reporter gene, whereas the remaining plasmids also contain CTA1 promoter fragments as follows: pMF14, −226 to −156 nucleotides upstream of the ATG start site; pMF17, −184 to −156 nucleotides; and pMF22, −214 to −184 nucleotides (21).

performed on labeled UAS<sub>1</sub>CTA1 using a recombinant Adr1p-LacZ fusion protein (20). The results demonstrated that the Adr1p-specific complex (16, 20) formed (Fig. 2D, lane 10) could be competed by adding excess UAS<sub>1</sub>POX1 but not POX1 ORE (lanes 14 and 15). The addition of excess UAS<sub>1</sub>CTA1 or UAS<sub>1</sub>ADH2 but not SP<sub>S</sub>19 ORE, gave a similar pattern of competition (lanes 11–13). Hence, it was reasoned that despite the ORE incorporated into it, the canonical UAS<sub>1</sub>POX1 represented a bona fide Adr1p element. The results also revealed an apparent intensification of the Adr1p signal in lanes containing SP<sub>S</sub>19 ORE or POX1 ORE competitor DNA (lanes 13 and 15) compared with the situation without competition (lane 10). However, this signal enhancement probably did not indicate an ORE-specific transactivation of UAS1 binding, because previous EMSAs (16) have shown that the addition of an ORE-less competitor DNA (BUFFAL) (34) also had a similar effect.

A Combination of ORE and UAS1 Is Required for Full Transcriptional Induction of CTA1—The prevalence of closely associated OREs and UAS1s among genes involved in peroxisomal processes has been reported previously (16). This raised the issue of whether the two elements cooperate to act as a transcriptional enhancer. The promoter elements in POX1 and PEX11 were deemed inappropriate for examining this potential cooperation, because in the former promoter the superimposition of the two elements is unique to this gene, whereas in the promoter of the latter gene the sequence overlapping the ORE is not a canonical UAS1.

Instead, we used the CTA1 promoter (Fig. 3A) to study the possible interaction between the two elements because (i) it contains a UAS1/ORE overlap, (ii) mutating either Pip2p or Adr1p did not lead to a complete loss of induced expression (Fig. 1B), which should facilitate analysis of the combined effect of both factors, and (iii) activities of reporter genes based on dissected CTA1 promoter fragments can be measured simultaneously with those of native catalase A using a convenient assay. UAS<sub>1</sub>CTA1 resembles the cognate sequence in the promoter of ADH2 (Fig. 3B) in that both elements contain a second half-site at their 5′ end (Fig. 3, A and B, underlined). The minimal sequences in UAS<sub>1</sub>CTA1 and UAS<sub>1</sub>ADH2 sufficient to form protein-DNA complexes with Adr1p are indicated (Fig. 3, A and B, brackets above sequences). By inserting the minimal UAS<sub>1</sub>CTA1, the isolated CTA1 ORE, or a stretch of DNA containing both elements (Fig. 3C) into a basal CYC1-lacZ reporter gene, it was possible to examine these promoter regions either as dissected or combined elements.

Oleic acid-induced yeast cells harboring a single copy of each of these integrative constructs were assayed for β-galactosidase and catalase activities. The results demonstrated that wild-type cells expressed the pMF14 reporter gene (containing both UAS1 and ORE) to at least a 4-fold greater extent than reporter genes consisting of either UAS1 (pMF17) or ORE (pMF22) alone (Table I). Oleic acid-dependent activation of pMF14 relied on both Adr1p and Pip2p, because a mutation of either gene reduced the levels of β-galactosidase activities to less than a fifth of the levels reached in the wild-type strain. This reduction appeared to be exacerbated in the adr1Δpip2Δ double mutant. Catalase activities measured in the same extracts substantiated the effect of both the adr1Δ and pip2Δ mutations on the expression of native CTA1 on oleic acid. Expression of the UAS<sub>1</sub>CTA1-driven reporter (pMF17) was effectively abolished in the adr1Δ mutant compared with the wild type, but also in the pip2Δ mutant only a residual activity was maintained. A similar situation was observed using the CTA1 ORE-containing reporter (pMF22) in that its expression was very low in the pip2Δ mutant and only partially retained in the adr1Δ mutant (Table I).

On ethanol, Adr1p predominated over Pip2p-Oaf1p in regulating CTA1, as judged by both types of enzyme activities (Table I). This was in agreement with current understanding of Pip2p-Oaf1p function that stipulates it is inactive in the absence of fatty acids (37). However, on oleic acid CTA1 ORE and UAS1CTA1 acted in synergy to enhance CTA1 expression. This indicated that a close association of these two elements in other dually regulated genes such as POX1 and potentially also in PEX11 might also act synergistically.
Acid-specific Pip2p-Oaf1p transcription factor and the more general sensor of less-favored carbon sources, Adr1p.

This postulate must take into account dual occupancy of the two transcription factors in such promoters where ORE and UAS1 overlap. Pip2p-Oaf1p can bind and weakly activate single half-sites within ORES (21, 35). Similarly, Adr1p can bind each of the two half-sites in UAS1 independently of each other (12). Hence, it is possible that not all of the contact sites within the corresponding palindromes are occupied simultaneously by Pip2p-Oaf1p and Adr1p in such overlapping element arrangements.

Finally, analysis of the Pip2 promoter revealed a sequence overlapping the Pip2 ORF with some semblance to UAS1, at position −124 (CTCCGGAGN³CTCCAA). In addition, the OAF1 promoter was also found to contain a potential UAS1 (CTCCAGN²CTGGGG) at position −392. Adr1p might turn out to influence the transcription of Pip2 and/or OAF1, thereby adding another layer of (indirect) control over genes subordinate to Pip2-Oaf1p. Further work is required to determine whether in this way Adr1p heads the transcriptional hierarchy leading to oleic acid induction and peroxisome proliferation.

REFERENCES

1. Moser, H. W. (1991) Clin. Biochem. 24, 343–351
2. Lanegrø, P. B. (1985) J. Neurogenet. Exp. Neurol. 54, 720–725
3. Chang, C.-C., South, S., Warren, D., Jones, J., Moser, A. B., Moser, H. W., and Gould, S. J. (1998) J. Cell. Physiol. 181, 229–234
4. Van Roermund, C. W., Tabak, H. F., Van Den Berg, M., Wanders, R. J., and Hettema, E. H. (2000) J. Cell. Biol. 150, 489–498
5. Simon, M., Adam, G., Ratapaz, W., Spevak, W., and Ruis, H. (1993) Mol. Cell. Biol. 11, 699–704
6. Künau, W. H., Demms, V., and Schulz, H. (1995) Prog. Lipid Res. 34, 267–242
7. Veenhuis, M., Matesiowlski, M., Kuna, W.-H., and Harder, W. (1987) Yeast 3, 72–84
8. Rottensteiner, H., Kal, A. J., Filipits, M., Binder, M., Hamilton, B., Tabak, H. F., and Ruis, H. (1996) EMBO J. 15, 2924–2931
9. Luo, Y., Karpiech, I. V., Kohanski, R. A., and Small, G. M. (1996) J. Biol. Chem. 271, 12068–12075
10. Simon, M. M., Pavlik, P., Hartig, A., Binder, M., Ruis, H., and Krimkevich, Y. I., Lark, E. H., and Denes, M. M. (1991) Mol. Gen. Genet. 230, 249–256
11. Ciriacy, M. (1975) Mol. Gen. Genet. 138, 157–164
12. Craciun, M. (1975) Mol. Gen. Genet. 138, 157–164
13. Cheng, C., Kacheryovsky, N., Dombek, K. M., Camier, S., Thukral, S. K., Rham, E., and Young, E. T. (1994) Mol. Cell. Biol. 14, 3824–3832
14. Simon, M., Binder, M., Adam, G., Hartig, A., and Ruis, H. (1992) Yeast 8, 363–369
15. Izgual, J. C., and Navarro, B. (1996) Mol. Cell. Biol. 232, 446–455
16. Gurvitz, A., Rottensteiner, H., Kilpeläinen, S. H., Hartig, A., Hiltunen, J. K., Binder, M., Dawes, I. W., and Hamilton, B. (1997) J. Biol. Chem. 272, 22140–22147
17. Gurvitz, A., Wabnegger, L., Rottensteiner, H., Dawes, I. W., Hartig, A., and Hamilton, B. (2000) Mol. Cell. Biol. Res. Commun. 4, 81–89
18. Dradowska, A., Dignard, D., Malewicz, R., and Thomas, D. Y. (1998) Gene (Amst.) 88, 247–252
19. Erdmann, R., and Bleidel, G. (1998) J. Cell. Biol. 139, 509–523
20. Marshall, P. A., Krimkevich, Y. I., Lark, E. H., Dyer, J. M., Veenhuis, M., and Goodman, J. M. (1995) J. Cell Biol. 129, 345–355
21. Eisen, A., Taylor, W. E., Blumberg, H., and Young, E. T. (1988) Mol. Cell. Biol. 8, 4552–4556
22. Filipits, M., Simon, M. M., Ratapaz, W., Hamilton, B., and Ruis, H. (1993) Gene (Amst.) 132, 49–55
23. Rottensteiner, H., Kal, A. J., Hamilton, B., Ruis, H., and Tabak, H. F. (1997) Eur. J. Biochem. 247, 77–82
24. Gurvitz, A., Rottensteiner, H., Hiltunen, J. K., Binder, M., Dawes, I. W., Ruis, H., and Hamilton, B. (1997) Mol. Microbiol. 26, 675–685
25. Jander, E. W. (1977) Genetics 83, 23–33
26. Yaffe, M. P., and Schatz, G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4819–4823
27. Erdmann, R., and Kunau, W.-H. (1999) Yeast 19, 1173–1182
28. Miller, J. H. (1973) J. Biol. Chem. 247, 3525–3535
29. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
30. Palosaari, P. M., Kilponen, J. M., Sormunen, R. T., Hassinen, I. E., and Hamilton, B. (2000) Mol. Gen. Genet. 262, 481–492
31. Laemmli, U. K. (1970) Nature 221, 497–500
32. Gurvitz, A., Hamilton, B., Hartig, A., Ruis, H., and Rottensteiner, H. (1999) Mol. Gen. Genet. 262, 481–492

DISCUSSION

Yeast cells devoid of Adr1p fail to utilize oleic acid as a sole carbon source or to expand their peroxisomal compartment (5). In the present study, the probable cause for this dual phenotype was revealed to be a defect in the expression of Pox1p and Pex11p. Although both FOX2 (encoding peroxosomal multifunctional enzyme type 2) (38) and POT1/FOX3 (encoding peroxosomal thiolase) (39, 40) have been shown previously to be influenced by Adr1p (5, 14), it was hitherto not clear to what extent the carbon flux through β-oxidation would be perturbed in adr1Δ mutant cells. This uncertainty was highlighted by the detection of relatively large amounts of Pot1p/Fox3p in adr1Δ cells compared with cells devoid of Pip2p-Oaf1p (Fig. 1A). However, unlike the situation with Pot1p/Fox3p, Adr1p was shown here to regulate Pox1p expression very tightly. This strict dependence of the β-oxidation spiral on Adr1p explains why adr1Δ mutants fail to degrade oleic acid.

Adr1p control was also demonstrated for PEX11, as determined by transcriptional analysis and immunoblotting. A direct role for Adr1p in regulating PEX11 remains to be demonstrated, because in contrast to POX1, the PEX11 promoter does not contain a canonical UAS1. Nevertheless, because of the occurrence of an ORE-overlapping sequence in the PEX11 promoter with only one mismatch to the UAS1 consensus, it is at least plausible that Adr1p might bind the PEX11 promoter. The possibility that the tandem array of UAS1 half-sites in the promoter spiral on Adr1p explains why adr1Δ mutants fail to degrade oleic acid.

Mutant cells lacking Pex11p or Pox1p resemble each other in that their peroxisomes appear enlarged, albeit reduced in number (4), and it has been suggested that this shared phenotype is caused by the absence of a putative metabolic signal generated via medium-chain fatty acid β-oxidation. A significant reduction (5-fold) in peroxisome abundance is also observed in human cell lines lacking peroxisomal acyl-CoA oxidase (3). By extrapolating this situation to yeast adr1Δ cells defective in peroxisomal acyl-CoA oxidase (Pox1p) activity, the peroxisome proliferation phenotype of adr1Δ cells might also turn out to be secondary to blocked β-oxidation (4). However, unlike the enlarged peroxisomes observed in pox1Δ or pex11Δ cells, the few peroxisomes seen in adr1Δ and pip2Δ mutant cells are unusually small. We propose that the reason for the small peroxisomes in these latter two mutant strains is because they contain less matrix enzymes and membrane proteins compared with wild-type peroxisomes, because of an absence of bulk transcription mediated by Adr1p and Pip2p-Oaf1p.

Using a basal CYC1-lacZ reporter gene, the significance of overlapping UAS1/ORE elements prevalent among oleic acid-inducible genes was addressed. This revealed that the combined potential for transcriptional activation of the isolated CTCA1 ORF and UAS1/CTCA1 was about 2.5-fold less than that conferred on the basal promoter by the arrangement found in the native promoter. This indicated synergy between the respective Pip2p-Oaf1p and Adr1p transcription factors. The argument for synergy was further supported by the observation that expression of the ORE reporter (pMF22) in the adr1Δ mutant or the UAS1 reporter (pMF17) in the pip2Δ mutant was less compared with that in the wild-type strain. It is attractive to postulate that for efficient expression of certain oleic acid-regulated genes, an enhancerosome-like (42) transient complex might be formed in vivo between the oleic

acid-specific Pip2p-Oaf1p transcription factor and the more general sensor of less-favored carbon sources, Adr1p.
Adr1p Regulates POX1 and PEX11

35. Einerhand, A. W., Kos, W. T., Distel, B., and Tabak, H. F. (1993) Eur. J. Biochem. 214, 323–331
36. Grauslund, M., Lopes, J. M., and Rønnow, B. (1999) Nucleic Acids Res. 27, 4391–4398
37. Baumgartner, U., Hamilton, B., Piskacek, M., Ruis, H., and Rottensteiner, H. (1999) J. Biol. Chem. 274, 22208–22216
38. Hiltunen, J. K., Wenzel, B., Beyer, A., Erdmann, R., Fosså, A., and Kunau, W.-H. (1992) J. Biol. Chem. 267, 6646–6653
39. Igual, J. C., Matallana, E., Gonzalez-Bosch, C., Franco, L., and Perez-Ortin, J. E. (1991) Yeast 7, 379–389
40. Einerhand, A. W., Voorn-Brouwer, T. M., Erdmann, R., Kunau, W.-H., and Tabak, H. F. (1991) Eur. J. Biochem. 200, 113–122
41. Pavlik, P., Simon, M., Schuster, T., and Ruis, H. (1993) Curr. Genet. 24, 21–25
42. Merika, M., and Thanos, D. (2001) Curr. Opin. Genet. Dev. 11, 205–208
Saccharomyces cerevisiae Adr1p Governs Fatty Acid β-Oxidation and Peroxisome Proliferation by Regulating POXI and PEX11

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