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## A heterodimer of the Zn<sub>2</sub>Cys<sub>6</sub> transcription factors Pip2p and Oaf1p controls induction of genes encoding peroxisomal proteins in *Saccharomyces cerevisiae*

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In the yeast *Saccharomyces cerevisiae*, two transcriptional activators belonging to the Zn<sub>2</sub>Cys<sub>6</sub> protein family, Pip2p and Oaf1p, are involved in fatty-acid-dependent induction of genes encoding peroxisomal proteins. This induction is mediated via an upstream activation sequence called the oleate-response element (ORE). DNA-bandshift experiments with ORE probes and epitope-tagged proteins showed that two binary complexes occurred: in wild-type cells the major complex consisted of a Pip2p · Oaf1p heterodimer, but in cells in which Oaf1p was overexpressed an Oaf1p homodimer was also observed. The genes encoding Oaf1p and Pip2p were controlled in different ways. The *OAF1* gene was constitutively expressed, while the *PIP2* gene was induced upon growth on oleate, giving rise to positive autoregulatory control. We have shown that the Pip2p · Oaf1p heterodimer is responsible for the strong expression of the genes encoding peroxisomal proteins upon growth on oleate. Pip2p and Oaf1p form an example of a heterodimer of yeast Zn<sub>2</sub>Cys<sub>6</sub> zinc-finger proteins binding to DNA.

**Keywords:** *PIP2*; *OAF1*; peroxisome; oleate-response element; *Saccharomyces cerevisiae*.

Peroxisomes are organelles present in almost all eukaryotic cells. Depending on the species they may harbour enzymes for diverse catabolic and anabolic processes, such as the  $\beta$ -oxidation of fatty acids and the first steps leading to ether-phospholipid synthesis (Van den Bosch et al., 1992; Reddy and Mannaerts, 1994). In the yeast *Saccharomyces cerevisiae* peroxisomes are the exclusive site for  $\beta$ -oxidation of fatty acids. When cells are grown on glucose medium, peroxisomal functions are less in demand and transcription of genes encoding peroxisomal proteins is repressed. On non-fermentable carbon sources such as ethanol or glycerol, derepression occurs. Full induction takes place when cells are grown on the fatty acid oleate as sole carbon source (Veenhuis et al., 1987). Induction of many of these genes is exerted via an upstream activation sequence called the oleate-response element (ORE), an inverted repeat of CGG triplets with a spacing of 15–18 nucleotides (CGGN<sub>15–18</sub>CCG) (Einerhand et al., 1993; Filipits et al., 1993). ORE have been found in upstream regions of genes encoding peroxisomal matrix proteins, such as fatty acyl-CoA oxidase (Fox1p), thiolase (Fox3p), catalase A (Cta1p) and malate dehydrogenase (Mdh3p), in upstream regions of genes encoding certain peroxisomal membrane proteins, such as Pex11p and Pat1p, and in the

upstream region of the gene encoding the transcriptional activator Pip2p (Wang et al., 1994; Einerhand et al., 1993; Filipits et al., 1993; Hiltunen et al., 1992; Erdmann and Blobel, 1995; Hettema et al., 1996; Distel et al., 1996; Rottensteiner et al., 1996).

Pip2p is a protein essential for induction of peroxisomal matrix proteins and for the proliferation of the peroxisomal compartment itself. Pip2p interacts directly with the ORE and mediates oleate-specific induction of ORE-dependent transcription (Rottensteiner et al., 1996). A second transcription factor, Oaf1p, was purified and cloned on the basis of its interaction with the *FOX1* ORE (Luo et al., 1996). Mutants of the *PIP2* or *OAF1* genes failed to grow on oleate as sole carbon source, and cell extracts of deletion strains showed no binding activity towards ORE in a DNA-bandshift assay. The *PIP2* and *OAF1* genes encode proteins belonging to the Zn<sub>2</sub>Cys<sub>6</sub> protein family of fungal transcriptional activators. Several members of this family have been shown to bind to palindromic CGG triplets, spaced by a certain number of residues, which defines the binding site for a particular member of the family. For instance, palindromic CGG triplets spaced by 11 bp define a binding site for Gal4p, while a 6-bp spacing defines the Ppr1p-binding site. The well-studied members of this typical fungal transcription-factor family of 56 reported Zn<sub>2</sub>Cys<sub>6</sub> proteins interact with their DNA-binding sites as homodimers. Gal4p, Ppr1p, Put3p, Cha4p and Tea1p bind to palindromic CGG repeats (Reece and Ptashne, 1993; Marmorstein and Harrison, 1994; Holmberg and Schjerling, 1996; Gray and Fassler, 1996); Leu3p and Pdr3p bind to everted CGG repeats (Hellauer et al., 1996), and Hap1 binds to directly repeated CGG triplets (Zhang and Guarente, 1994).

The high similarity of Pip2p and Oaf1p and the similar phenotypes of *pip2Δ* and *oaf1Δ* strains suggest that the proteins are

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Abbreviations. HA, haemagglutinin epitope; GST, glutathione S-transferase; ORE, oleate-response element; PPAR, peroxisome proliferator-activated receptor.

Note. H. Rottensteiner and A. J. Kal contributed equally to this paper.

involved in the same process required for activation of genes encoding peroxisomal proteins. Therefore, we carried out experiments to study in more detail the involvement of Pip2p and Oaf1p in the induction of genes encoding peroxisomal proteins. Here we show that the *PIP2* and *OAF1* genes are regulated differentially: the *PIP2* gene is positively autoregulated, while the *OAF1* gene is constitutively expressed. In the absence of Pip2p, overexpressed Oaf1p binds ORE, probably as a homodimer. The most important finding of this investigation is that under physiological circumstances Pip2p and Oaf1p form a functional heterodimer that binds to ORE.

## MATERIALS AND METHODS

**Yeast strains, media and culture conditions.** The following *S. cerevisiae* strains were used in this study: BJ1991 (*MAT $\alpha$* , *leu2 trp1 ura3-52 pep4-3 prb1-1122*) (Jones, 1977); and MF24-6x [*MAT $\alpha$* , *leu2 his3 trp1 ctt1-1 gal2 URA3:::(CTA1-184/-198)<sub>6</sub>-CYC1-lacZ*] (Rottensteiner et al., 1996). BJ1991*pip2 $\Delta$* , BJ1991*oaf1 $\Delta$* , BJ1991*pip2 $\Delta$ oaf1 $\Delta$* , MF24-6x *pip2 $\Delta$* , MF24-6x *oaf1 $\Delta$*  and MF24-6x *pip2 $\Delta$ oaf1 $\Delta$*  were derived from the two parental strains by gene disruption of the *PIP2* or *OAF1* loci (Rottensteiner et al., 1996; this study).

For expression of Pip2p and the triple-haemagglutinin-epitope(HA)-tagged variant Pip2p-HA<sub>3</sub> under control of the *TP11* promoter, the required strains were transformed with centromeric plasmids pTPI1-PIP2 and pTPI1-PIP2HA3, respectively. For expression of Oaf1p and the variant tagged with nine Myc epitopes, Oaf1p-Myc<sub>9</sub>, under control of the *ADH2* promoter, integrative plasmids pADH2-OAF and pADH2-OAFMYC9T, respectively, were digested with *Bst*XI and integrated in the *TRP1* locus of the required strains. BJ1991*pip2 $\Delta$ oaf1 $\Delta$*  expressing Pip2p-HA<sub>3</sub> and Oaf1-Myc<sub>9</sub> was prepared by transformation with centromeric plasmid pTPI1-PIP2HA3 and integration of *Stu*I-digested pADH2-OAFMYC9U into the *URA3* locus.

For catalase A assays,  $\beta$ -galactosidase assays, luciferase assays and bandshift assays, cells were cultured overnight on minimal medium containing 0.67% yeast nitrogen base (Difco), 2% glucose and 20 mg/l amino acids as required. They were then incubated in minimal medium containing 0.3% glucose for 10 h, then in yeast extract peptone containing either 2% glucose, 2% ethanol, or 0.2% oleic acid/0.02% Tween-80 for 18 h until the cultures reached an  $A_{600}$  of 1.0–1.5.

**Cloning procedures and gene disruptions.** Standard techniques for DNA manipulations were used throughout this study (Sambrook et al., 1989). Yeast transformation was carried out as described (Ito et al., 1983).

To clone the *FOX1* ORE, annealed oligonucleotides (5'-AAT TCA TTT ACG GTA TTA GTT GAT TAA ACT CCG AAG CG-3') and (5'-GAT CCG CTT CGG AGT TTA ATC AAC TAA TAC CGT AAA TG-3') were ligated into vector pBluescriptSK(+) digested with *Eco*RI and *Bam*HI, resulting in plasmid pAK77. The sequences of the cloned oligo nucleotides were verified by sequencing.

The *OAF1* gene was amplified by PCR with genomic DNA from BJ1991 and the primers OAF1-START (5'-TTT TCT AGA TCC ATG GTC GAA AAT AGT ACG CAG-3') and OAF1-STOP (5'-TTT AAG CTT TTA ATT GAT GAA ATG AAT AGG TAG G-3'). The PCR fragment was digested with *Xba*I and *Hind*III and cloned into pUC19*Ava*I digested with *Xba*I and *Hind*III, resulting in pAK56.

To disrupt the *OAF1* gene, a 1.2-kb *Xho*I fragment from pAK56 encoding Oaf1p amino acids 424–810 was replaced by a 2.0-kb *Sal*I fragment of pJJ252 (Jones and Prakash, 1990) containing the *LEU2* gene, resulting in pAK83. The *oaf1 $\Delta$*  strains

were obtained after transformation of an *Nco*I–*Hind*III fragment of pAK83 and screening for leucine prototrophs.

To disrupt the *PIP2* gene in strains BJ1991, BJ1991*oaf1 $\Delta$* , MF24-6x and MF24-6x *oaf1 $\Delta$*  the EUROFAN short flanking homology method was chosen (Wach et al., 1994). The *PIP2* gene was replaced by the KanMX4 resistance marker by transformation of a PCR fragment obtained from a PCR on plasmid pFA6a-KanMX4 with primers *PIP2* S1 (5'-AAT ATC ACT TTC TGA TCT TGC TGA ACA AAA TTT CTT CGA TAA AAG CGT ACG CTG CAG GTC GAC-3') and *PIP2* S2 (5'-TGA AGT GGC CGT ATA CAT CTC AGG AAA ACT GGA AAA GAC TTC CTA ATC GAT GAA TTC GAG CTC G-3'). All gene deletions in this study were verified by Southern blot analysis (data not shown). The PCR-generated *pip2 $\Delta$*  strains were similarly deficient for oleate growth and catalase A expression as the *pip2 $\Delta$*  strains reported previously (Rottensteiner et al., 1996) and were used throughout this work.

The Pip2p overexpression construct pTPI1-PIP2 contains a 3.0-kb *Nde*I–*Sal*I fragment from pSKMP3 (Rottensteiner et al., 1996) cloned into the *Nde*I/*Sal*I-digested pUH60 (unpublished results), a derivative of pGA1840 (Gartner et al., 1992) with an additional *Sal*I site 560 bp prior to the *Bam*HI site.

For construction of pTPI1-PIP2HA3 a *Not*I adaptor consisting of oligo nucleotides *Not*I-1 (5'-CATGAGCGGCCGCT-3') and *Not*I-2 (5'-CATGAGCGGCCGCT-3') was ligated into the *Nco*I site of pSKMP3 (Rottensteiner et al., 1996) resulting in pSKMP3-*Not*I. The newly generated *Not*I site was used to insert a 111-bp *Not*I triple-HA-epitope cassette (Tyers et al., 1993), resulting in pSKMP3-HA3. The 1.2-kb *Sal*I–*Bgl*III fragment of pTPI1-PIP2 was replaced by the 1.3-kb *Sal*I–*Bgl*III fragment of pSKMP3-HA3.

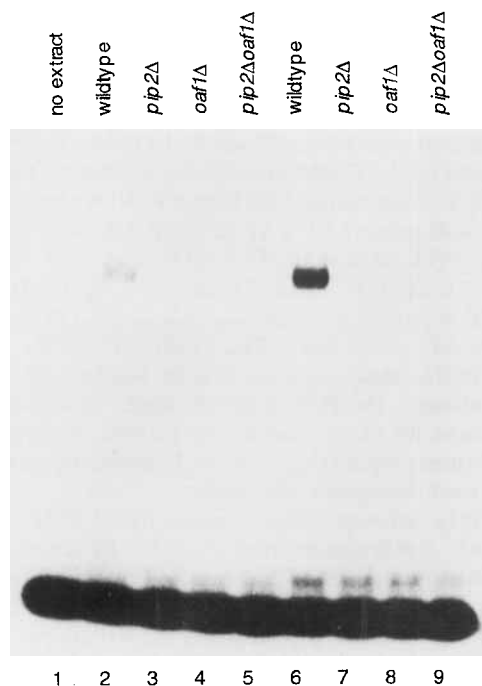
The Oaf1p overexpression plasmid pADH2-OAF1 was generated by inserting the 3.2-kb *Hind*III–*Nco*I fragment from pAK56 and a PCR product containing the 744-bp *Nco*I–*Eco*RI fragment of the *ADH2* promoter into an *Eco*RI/*Hind*III-digested YIplac204 (Gietz and Sugino, 1988). This PCR was performed with genomic DNA and the oligo nucleotides ADH2-1 (5'-AGA ATT CAT CAA ATT GTG GGA TAC TCC C-3') and ADH2-2 (5'-ACC ATG GTT GTG TAT TAC GAT ATA GTT AAT AG-3').

For construction of pADH2-OAF1MYC9T a *Not*I adaptor (see above) was ligated in the *Nco*I site of pADH2-OAF1. The resulting *Not*I site was used to insert a 350-bp *Not*I cassette containing nine copies of the Myc epitope described by Piatti et al. (1996). The URA3-selectable pADH2OAF1MYC9U was cloned by ligating the 4.5-kb *Hind*III–*Nar*I fragment of pADH2-OAF1MYC9T into *Hind*III/*Nar*I-digested YIplac211 (Gietz and Sugino, 1988).

The reporter construct pPIP2lacZ contains 1044 bp of the *PIP2* promoter fused upstream of *lacZ* and was constructed by ligation of a 1.1-kb *Eco*RV–*Hind*III fragment from pPIP2-9 (Rottensteiner et al., 1996) into a *Sma*I/*Hind*III-digested pYE353 (Myers et al., 1986).

The reporter construct pOAF1lacZ contains 392 bp of the *OAF1* promoter fused upstream of *lacZ* and was constructed by ligation of a 700-bp *Eco*RI PCR fragment generated with the oligo nucleotides OAF1-prom (5'-AAT TGT CGA CGG TTT TGT CAG TGA TGG CGG-3') and OAF1-rev (5'-CGC GTT CGC CGT CAA TGC CGG-3') into an *Eco*RI-digested pYE358R (Myers et al., 1986).

**Enzyme activities.** Activities of  $\beta$ -galactosidase, catalase A and luciferase were measured as described (Rottensteiner et al., 1996). Protein concentrations were measured by the method of Bradford (1976). All enzyme activities presented are the average of results obtained in at least two experiments.



**Fig. 1.** The ORE-binding complex is present in wild-type cell extracts but absent in *pip2Δ*, *oaf1Δ* or *pip2Δoaf1Δ* cell extracts. Extracts were tested in the DNA-bandshift assay with the *FOX3* ORE as a probe. Lane 1, no extract added. Extracts of yeast cells grown on glucose (lanes 2–5) or oleate (lanes 6–9).

**Antibodies and western blotting.** Tissue culture supernatants containing anti-Myc and anti-HA (cell lines 9E10 and 12CA5, respectively) mouse mAb were a gift from Anton Gartner. For western blotting, protein extracts were separated by SDS/PAGE and blotted to nitrocellulose filters. Secondary anti-(mouse horseradish peroxidase) antibodies and SuperSignal (Pierce) were used to visualise HA-tagged and Myc-tagged proteins.

**Bandshift assays.** Bandshift assays, DNA labelling and isolation of the *FOX3* ORE and *CTA1* ORE were described previously (Rottensteiner et al., 1996). The *FOX3* ORE probe was 85 bp in length, the *FOX1* and *CTA1* ORE probes were 38 bp and 37 bp, respectively. The *FOX1* ORE was isolated from pAK77 by digestion with *EcoRI* and *BamHI*. Bandshift reactions with 2- $\mu$ l protein samples (30  $\mu$ g) and 0.2 ng  $^{32}$ P-labelled DNA probes were carried out in the presence of 4 mM Tris/HCl, pH 8, 100 mM NaCl, 4 mM MgCl<sub>2</sub>, 25 ng/ $\mu$ l poly[d(I-C) · d(C-I)] and 4% glycerol in 20  $\mu$ l. Bandshift reactions were incubated for 25 min at room temperature. Where protein · DNA complexes were supershifted, 1  $\mu$ l mAb against HA (diluted 10 fold) or Myc-epitope (undiluted) was added to the bandshift reactions after 5 min incubation and reactions were further incubated for 20 min at room temperature.

## RESULTS

**Pip2p and Oaf1p are both required for the oleate-induction process and for ORE binding.** We constructed a *pip2Δoaf1Δ* double-deletion strain, which showed essentially the same phenotype as the strains with the single *pip2Δ* or *oaf1Δ* deletions; no growth deficiencies were found on glucose, ethanol, glycerol or acetate media, but growth on oleate medium was impaired (data not shown).

To study the DNA-binding activities towards ORE, we performed DNA-bandshift assays with the  $^{32}$ P-labelled *FOX3* ORE

**Table 1.** Determination of catalase A activity in wild-type and mutant cells. Catalase A activity in oleate-grown cells. Catalase A activity in wild-type cells was set at 100%.

Strain	Catalase A activity
	%
MF24-6x	100.0 $\pm$ 6.0
MF24-6x <i>pip2Δ</i>	26 $\pm$ 0.5
MF24-6x <i>oaf1Δ</i>	8.2 $\pm$ 1.6
MF24-6x <i>pip2Δoaf1Δ</i>	11.4 $\pm$ 1.9

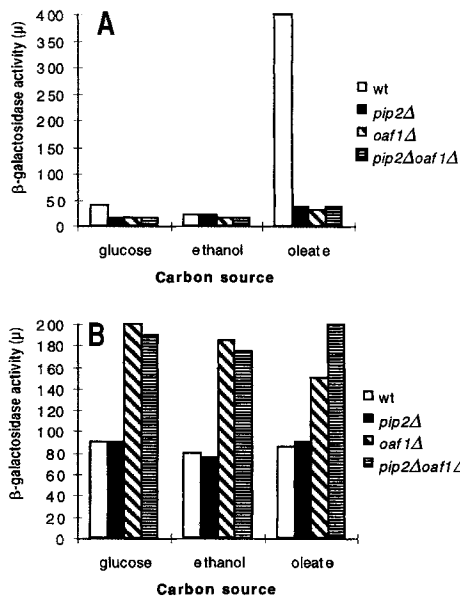
**Table 2.** Determination of luciferase activity in wild-type and mutant cells. Luciferase activity in oleate-grown cells. Luciferase activity in wild-type cells transformed with YTL985 was set at 100%.

Strain	Luciferase activity in cells transformed with	
	YTL985	YTLtt985
	%	
BJ1991	100.0 $\pm$ 6.6	6.8 $\pm$ 0.5
BJ1991 <i>pip2Δ</i>	12.2 $\pm$ 2.2	2.3 $\pm$ 0.7
BJ1991 <i>oaf1Δ</i>	6.5 $\pm$ 0.6	1.7 $\pm$ 0.1
BJ1991 <i>pip2Δoaf1Δ</i>	7.2 $\pm$ 0.4	2.2 $\pm$ 0.1

as a probe with extracts from wild-type, *pip2Δ*, *oaf1Δ* and *pip2Δoaf1Δ* cells grown on glucose or oleate (Fig. 1). The ORE-binding complex was present in extracts from wild-type cells grown on glucose and was induced in cells grown on oleate (Fig. 1), as described before (Rottensteiner et al., 1996). The ORE-binding complex was not present in extracts from *pip2Δ*, *oaf1Δ* and *pip2Δoaf1Δ* cells (lanes 3–5 and 7–9). The same results were obtained for ORE from *CTA1* and *FOX1* (data not shown).

Despite the similarities between *pip2Δ* and *oaf1Δ* strains, a subtle difference in phenotype was found when catalase A activity was measured in cells grown on oleate (Table 1). The amount of residual catalase A activity measured in *oaf1Δ* cells was about 2–3-fold lower than in the *pip2Δ* cells. Similar results were obtained when luciferase activities were measured in a different strain transformed with reporter plasmid YTL985 expressing the firefly luciferase gene driven by the *FOX3* promoter (Einerhand et al., 1991; Table 2). This indicated that Oaf1p in the absence of Pip2p could activate transcription of *CTA1* and *FOX3* genes at a low level. The difference between luciferase activities in *pip2Δ* and *oaf1Δ* cells was not detected when one of the CGG triplets of the ORE in the *FOX3* promoter was mutated [plasmid YTLtt985 (Einerhand et al., 1991)], which is consistent with previous conclusions that the ORE is critical in transcription activation by Pip2p and Oaf1p (Rottensteiner et al., 1996; Luo et al., 1996).

**Regulation of the *PIP2* and *OAF1* genes.** We reported previously that the promoter of the *PIP2* gene contains an ORE to which the Pip2p-containing complex can bind (Rottensteiner et al., 1996), and we suggested that Pip2p can regulate its own expression. Here we analysed this in more detail by means of a *PIP2-lacZ* reporter plasmid transformed into wild-type, *pip2Δ*, *oaf1Δ* and *pip2Δoaf1Δ* strains.  $\beta$ -Galactosidase activity was measured in extracts from cells growing on glucose, ethanol or oleate media (Fig. 2A). In wild-type cells grown on glucose or



**Fig. 2. Regulation of the *PIP2* and *OAF1* genes.** Reporter plasmids pPIP2lacZ (A) or pOAF1lacZ (B) were transformed into wild type, *pip2Δ*, *oaf1Δ* and *pip2Δoaf1Δ* cells.  $\beta$ -Galactosidase activity was measured after growth on the carbon source as indicated.

**Table 3. Catalase A activities in oleate-grown cells.** Determination of catalase A activities in oleate-grown wild-type cells and mutant cells expressing *PIP2* and *OAF1* genes from *TP11* and *ADH2* promoters, respectively. Catalase A activity in wild-type cells was set at 100%.

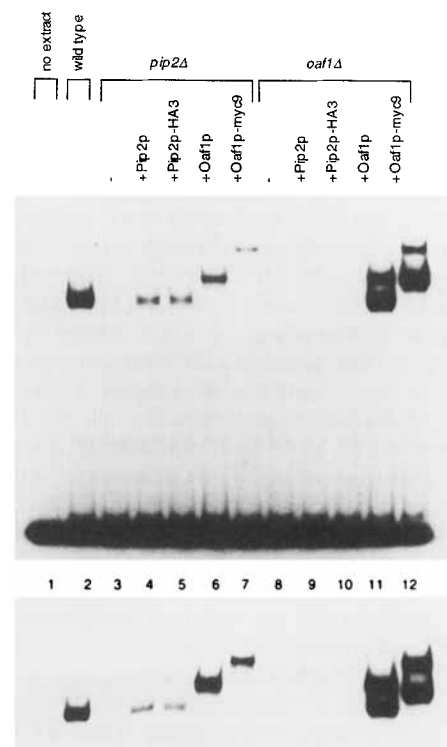
Strain	Catalase A activity
	%
MF24-6x	100.0 $\pm$ 6.0
MF24-6x <i>pip2Δ</i> <i>TP11-PIP2</i>	75.0 $\pm$ 7.1
MF24-6x <i>oaf1Δ</i> <i>TP11-PIP2</i>	6.8 $\pm$ 0.3
MF24-6x <i>oaf1Δ</i> <i>ADH2-OAF1</i>	139.0 $\pm$ 2.8
MF24-6x <i>pip2Δ</i> <i>ADH2-OAF1</i>	74.0 $\pm$ 5.2

ethanol,  $\beta$ -galactosidase was expressed at low levels and was induced 10–20-fold on oleate. In *pip2Δ*, *oaf1Δ* and *pip2Δoaf1Δ* this induction was absent. These results indicate that the *PIP2* gene is not only under control of Pip2p itself but is also dependent on the presence of Oaf1p.

To study whether or not the *OAF1* gene is controlled in a carbon-source-dependent manner, similar experiments as described above were performed with an *OAF1*-lacZ reporter plasmid (Fig. 2B). We found that the *OAF1* gene was constitutively expressed on all carbon sources tested and that the expression was independent of Pip2p. Deletion of the *OAF1* gene resulted in a twofold increase in the expression of the lacZ reporter, indicating that Oaf1p may have some negative effect on its own expression. That no ORE could be detected in the *OAF1* promoter is in accordance with the lack of oleate induction. The finding of a progressive increase in the specific ORE-complex formation upon induction on oleate (Fig. 1; Luo et al., 1996; Rottensteiner et al., 1996) is therefore due to the induction of *PIP2* expression.

#### Pip2p and Oaf1p are present in the ORE-binding complex.

We expressed Pip2p and Oaf1p from heterologous promoters to ensure expression on oleate medium, even in deletion strains. This was especially important for expression of the *PIP2* gene



**Fig. 3. Analysis of ORE-binding complexes.** Extracts from oleate-grown cells were tested in the DNA-bandshift assay with the *FOX3* ORE (upper panel) or the *FOX1* ORE (lower panel) as a probe. For the *FOX1* ORE, only the shifted complexes are shown. Lane 1, no extract added; lane 2, extract from wild-type cells; lanes 3–7, extracts from *pip2Δ* cells expressing Pip2p and Oaf1p and their epitope-tagged variants as indicated; lanes 8–12, extracts from *oaf1Δ* cells expressing Pip2p and Oaf1p and their epitope-tagged variants as indicated.

since its expression was low in the *oaf1Δ* strain. For Pip2p we applied the triose phosphate isomerase (*TP11*) promoter, and for Oaf1p the alcohol dehydrogenase II (*ADH2*) promoter. We also epitope tagged Pip2p with a triple-HA epitope ( $HA_3$ ) and Oaf1p with nine copies of a Myc epitope ( $Myc_9$ ). We subsequently tested all constructs for functionality (data not shown). The constructs complemented the mutant cells for growth on oleate plates and for catalase A activity. Moreover, the tagged versions were stably expressed in *pip2Δ* and *oaf1Δ* strains, as shown by western blotting (data not shown). In *pip2Δ* cells transformed with the *TP11-PIP2* construct, catalase A activity was restored to 75% of the wild-type level. Expression of Oaf1p in the *pip2Δ* and *oaf1Δ* strains restored catalase A activity to 74% and 139%, respectively, whereas no such enhanced levels were observed in *oaf1Δ* cells expressing Pip2p (Table 3). Apparently, Oaf1p when overexpressed could act independently of Pip2p in activation of at least some genes encoding peroxisomal proteins.

We exploited the *TP11*-promoter-driven and *ADH2*-promoter-driven *PIP2* and *OAF1* genes encoding the epitope-tagged versions of Pip2p and Oaf1p to analyse the composition of the protein-DNA complexes formed with ORE-containing oligonucleotides. We expressed the various proteins in *pip2Δ* and *oaf1Δ* cells and performed DNA-bandshift assays with the *FOX3* or *FOX1* ORE as probes (Fig. 3). Incubation of radiolabelled ORE DNA in extracts from wild-type cells grown on oleate resulted in formation of an ORE-binding complex (Fig. 3; complex A). Formation of this retarded complex was not observed in an extract from *pip2Δ* cells. When in *pip2Δ* cells Pip2p or its epitope-tagged derivative was expressed from the *TP11* promoter, formation of the complex was restored (Fig. 3).

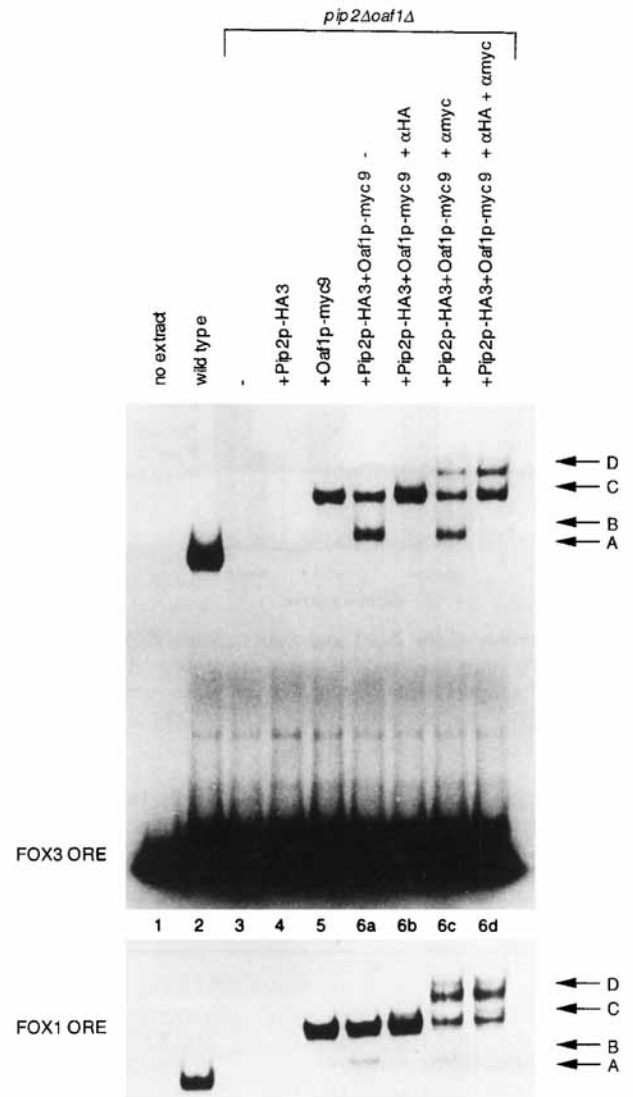
When Oaf1p was expressed in the *pip2A* strain at high levels from the *ADH2* promoter, the complex seen in extracts from wild-type cells was not restored. However, a different retarded complex was present that was located at a higher position in the gel than the wild-type complex (Fig. 3; complex B). Addition of the large Myc<sub>9</sub> tag to Oaf1p shifted this complex to an even higher position complex C. Because Pip2p was absent in these extracts, we suggest that complexes B and C consist of an Oaf1p homodimer. The homodimers of Oaf1p migrated at a different position compared with that of complex A in wild-type cells. Because the molecular masses of Pip2p (114.6 kDa) and Oaf1p (118.8 kDa) are almost equal, the different amino acid composition of Pip2p and Oaf1p and/or a different structure of the Oaf1p homodimer compared with that of complex A may explain this observation of different migration on the native gel.

The same set of incubations was carried out with protein extracts derived from an *oaf1Δ* strain. In *oaf1Δ* cell extracts no complex is visible (Fig. 3). Expression of Pip2p or Pip2p-HA<sub>3</sub> in the *oaf1Δ* strain did not restore formation of the complex, despite that the expressed Pip2p was present in these cells as shown by western blotting (data not shown). We conclude that expression of Pip2p did not lead to a homodimer complex of Pip2p capable of binding ORE as we observed for Oaf1p (compare lanes 9 and 10 with 6 and 7). Overexpression of Oaf1p in the *oaf1Δ* strain (i.e. in the presence of Pip2p) resulted in the formation of two complexes (complexes A and B). The complex at the lower position (complex A) migrated to the same position as the complex seen in wild-type extracts. Formation of this complex was dependent on Pip2p and, therefore, probably consisted of a Pip2p · Oaf1p heterodimer. The higher complex (complex B) was identical to the Oaf1p homodimer in lane 6. Both complexes shifted to a higher position in the gel when protein extracts were used containing the Myc<sub>9</sub>-tagged Oaf1p (complexes A and B shift to D and C, respectively). This is consistent with the presence of Oaf1p in both complexes. Similar results were obtained with the *FOX1* ORE (Fig. 3).

To show that Pip2p and Oaf1p bind to ORE as a heterodimer, we expressed the tagged proteins in the *pip2Δoaf1Δ* double-deletion strain and tested whether the complexes observed could be influenced in their migration by incubation with mAb directed against the epitope tags when the *FOX3* and *FOX1* ORE were used as probes (Fig. 4). Addition of a protein extract from wild-type cells showed the ORE-binding complex (complex A), which was absent in the untransformed *pip2Δoaf1Δ* strain. When Pip2p-HA<sub>3</sub> was expressed alone in the *pip2Δoaf1Δ* strain no complex was observed. Expression of Oaf1p-Myc<sub>9</sub> resulted in formation of a complex higher up in the gel (complex C). When both tagged proteins were expressed, two complexes became visible (lane 6a).

The expression of both tagged proteins formed the basis for the identification of the proteins in the complexes. Addition of anti-HA mAb directed against Pip2p-HA<sub>3</sub> to the bandshift reaction supershifted only the lower complex (complex B) to a higher position in the gel (complex C), coinciding with the upper band, which indicates that Pip2p-HA<sub>3</sub> is present in complex B. *pip2A* cells expressing only Pip2p-HA<sub>3</sub> lacked the upper complex, and for the lower complex the same shift was observed upon addition of the anti-HA mAb (data not shown), excluding the possibility that the anti-HA mAb interfere with ORE recognition by Pip2p-HA<sub>3</sub>.

When Oaf1p-Myc<sub>9</sub> was expressed at a low level from the *OAF1* promoter instead of the *ADH2* promoter, only complex B was present and addition of anti-Myc mAb supershifted complex B to a position coinciding with complex C (data not shown). Addition of anti-Myc mAb to a bandshift reaction (lane 6a) supershifted both complexes B and C (lane 6c). Complex B was



**Fig. 4. Analysis of ORE-binding complexes by means of anti-HA and anti-Myc mAb.** Extracts from oleate-grown cells were tested in the DNA-bandshift assay with the *FOX3* ORE (upper panel) or the *FOX1* ORE (lower panel) as a probe. For the *FOX1* ORE, only the shifted complexes are shown. Lane 1, no extract added; lane 2, extract from wild-type cells; lanes 3–6, extracts from *pip2Δoaf1Δ* cells expressing Pip2p and Oaf1p and their epitope-tagged variants as indicated; lanes 6b–6d: extracts from *pip2Δoaf1Δ* cells expressing Pip2p-HA<sub>3</sub> and Oaf1p-Myc<sub>9</sub> as indicated, incubated with mAb against HA (lane 6b), the Myc epitope (lane 6c) or both (lane 6d).

shifted to a position coinciding with complex C, and complex C was shifted to complex D. Thus, this experiment revealed the presence of Oaf1p-Myc<sub>9</sub> in complexes B and C. A complete supershift with the anti-Myc mAb was not possible due to their limited concentration in the tissue-culture supernatants.

When both mAb were added, complex B was supershifted to complex C when anti-Myc or anti-HA mAb were bound. This complex was further supershifted to complex D when both antibodies bound simultaneously. Complex C was supershifted to complex D, to a comparable extent as in the presence of anti-Myc mAb (lane 6c). The resolution of the native bandshift gels was not sufficient to separate the high-molecular-mass complexes that form complex D (i.e. ORE DNA · Pip2p-HA<sub>3</sub> · anti-HA · Oaf1p-Myc<sub>9</sub> · anti-Myc and ORE DNA · Oaf1p-Myc<sub>9</sub> · Oaf1p-Myc<sub>9</sub> · anti-Myc). A complex of ORE DNA · Oaf1p-Myc<sub>9</sub> · anti-Myc · Oaf1p-Myc<sub>9</sub> · anti-Myc was not seen due to

either insufficient resolution of the bandshift gel or to the limited concentration of anti-Myc mAb in the tissue culture supernatants. From the observations we conclude that the upper complex (complex C) was an Oaf1p-Myc<sub>9</sub> homodimer and that the lower complex (complex B) was a heterodimer of Pip2p-HA<sub>3</sub> and Oaf1p-Myc<sub>9</sub>. Together, the experiments shown in Figs 3 and 4 allowed us to conclude that the complex present in wild-type cell extracts consisted of a heterodimer of Pip2p and Oaf1p. In extracts prepared from cells overexpressing Oaf1p an Oaf1p homodimer complex could be formed. When the relative amounts of the Pip2p · Oaf1p heterodimer and the Oaf1p homodimer binding to certain ORE were compared (Fig. 4) it appeared that the homodimer had a higher affinity for the *FOX1* ORE than for the *FOX3* ORE. This indicated that the type of ORE and the homodimeric and heteromeric complexes might influence promoter recognition. However, under physiological conditions in the presence of oleate, all Oaf1p was present in the heterodimer complex with Pip2p.

In a previous study (Rottensteiner et al., 1996) we reported that Pip2p was likely to bind ORE as a homodimer, based on gel-filtration experiments and binding of *Escherichia coli*-produced glutathione *S*-transferase(GST)-Pip2p-(1–179)-peptide in DNA-bandshift assays. The previous gel-filtration studies are in accordance with the present conclusion that Pip2p and Oaf1p are in a heteromeric complex, since Pip2p and Oaf1p are very similar in molecular mass (Pip2p 114.6 kDa; Oaf1p 118.8 kDa). The results from the GST-Pip2p fusion protein may in hindsight be explained by that GST is a dimeric protein (Ji et al., 1992). The GST part of the GST-Pip2p fusion protein might have been responsible for dimerisation, leading to functional ORE binding.

## DISCUSSION

Peroxisomes form a flexible compartment in the eukaryotic cell and their number may vary from one to more than a hundred. The number, volume and morphology of peroxisomes, and the extent of transcription of genes encoding peroxisomal proteins are determined largely by the differentiation state of the cells and by their environment. For instance, administration of certain xenobiotics, such as hypolipidemic drugs or plasticizers, results in proliferation of peroxisomes in rodent tissues (Lock et al., 1989). These compounds act via peroxisome proliferation-activating receptors (PPAR), transcription factors belonging to the nuclear hormone-receptor family. Studies on PPAR $\alpha$  knock-out mice showed that PPAR $\alpha$  is a crucial transcription factor involved in the control of the peroxisomal compartment size (Lee et al., 1995). The active DNA-binding complex consists of a heterodimer of PPAR and the retinoid-X receptor, which exerts its effect via peroxisome-proliferator-response elements (Schoonjans et al., 1996).

*S. cerevisiae* uses different types of transcription factors for the same purpose. Pip2p and Oaf1p have been described as transcription factors for the induction of many genes encoding peroxisomal proteins and for the enlargement of the peroxisomal compartment as a whole (Luo et al., 1996; Rottensteiner et al., 1996). The *PIP2* gene (YOR363c) is localised on chromosome XV and the *OAF1* gene (YAL051w) on chromosome I. They lie in regions that are likely to originate from an early genome duplication (Wolfe and Shields, 1997). Both proteins are highly similar and belong to the Zn<sub>2</sub>Cys<sub>6</sub> family of transcription factors, of which Gal4p and Ppr1p are well-known examples. Pip2p and Oaf1p bind to ORE present in the upstream region of many peroxisomal protein-encoding genes. Strains in which either the *PIP2* or *OAF1* gene is deleted show the same phenotype: inability to grow on the fatty acid oleate as sole carbon source.

Recently, Karpichev et al. (1997) reported that Pip2p (which they called Oaf2p) and Oaf1p can be coimmunoprecipitated, suggesting that heterodimeric complexes bound to ORE. Here we describe in more detail how Pip2p and Oaf1p function in the activation of promoters containing ORE. Upon addition of ORE-containing DNA to protein extracts derived from oleate-grown cells, a single shifted DNA · protein complex was formed (Fig. 1). By means of functional epitope-tagged derivatives of Pip2p and Oaf1p introduced into mutants deleted for their endogenous wild-type *PIP2* and/or *OAF1* genes, we could demonstrate that this shifted complex consisted of a heterodimer comprising Pip2p and Oaf1p (Figs 3 and 4). In singly deleted strains no evidence was found for the presence of Pip2p homodimers in the *oaf1Δ* strain, but in the *pip2Δ* strain a weak signal of a putative Oaf1p-homodimer-shifted complex was observed after long exposure of the gels (data not shown). Catalase A and luciferase measurements (Tables 1 and 2) suggested that Oaf1p alone may mediate a low level of *CTA1* and *FOX3* transcription. In a *pip2Δ* strain overexpressing Oaf1p, the presence of the Oaf1p homodimer could be demonstrated in DNA-bandshift experiments (Figs 3 and 4). This Oaf1p homodimer was transcriptionally active, as shown by measurement of catalase A activity in *pip2Δ* and *oaf1Δ* mutant strains overexpressing Oaf1p (Table 3).

Indications for the functional significance of the Pip2p · Oaf1p heterodimer and the Oaf1p · Oaf1p homodimer were obtained by studying the expression of the genes encoding the transcription factors (Fig. 2). This was carried out by replacing the corresponding reading frames with that of the gene encoding  $\beta$ -galactosidase and using this enzyme as a reporter for changes in gene expression. The *OAF1* gene was constitutively expressed under all conditions tested: glucose repression; ethanol derepression; and oleate induction. On the contrary, the *PIP2* gene was strictly controlled by carbon source; it was glucose-repressed and fully induced when cells were grown on oleate. This is consistent with the presence of an ORE in the promoter of the *PIP2* gene (Rottensteiner et al., 1996) and our observations that Pip2p stimulated its own synthesis. Therefore, we propose that the increasing amount of Pip2p in oleate-induced cells recruits the Oaf1p in the heterodimer that binds to the ORE. Combined with the results that heterodimer formation was preferred over Oaf1p-homodimer formation, we would like to suggest the following role for the individual transcription factors.

Under glucose conditions (i.e. non-inducing conditions for *PIP2*) the amount of Oaf1p exceeds that of Pip2p, and Oaf1p could exist as a homodimer. In addition to Pip2p, two Zn<sub>2</sub>Cys<sub>6</sub> proteins of unknown function and with high similarity to Oaf1p are present in *S. cerevisiae* (YLL054c and YCR106w). Therefore we cannot exclude the possibility that Oaf1p also forms a heterodimer with another Zn<sub>2</sub>Cys<sub>6</sub> protein, recognising a binding site different from the ORE. When cells grow on oleate as sole carbon source they induce expression of Pip2p, thus mobilising Oaf1p into the heterodimeric complex at the expense of the homodimer Oaf1p complex. This apparent preference for heterodimer formation is similar to the behaviour of certain mammalian transcription factors, such as Fos and Jun. Fos and Jun contain single leucine zippers, a protein motif responsible for mediating protein interactions. The favoured interaction is between the zippers of Fos and Jun, resulting in Fos · Jun heterodimer formation. Self-interaction is observed between the zippers of Jun giving rise to Jun homodimer formation, while Fos only weakly interacts with itself (O'Shea et al., 1989, 1992; Smeal et al., 1989).

Combinatorial interactions enlarge the repertoire of transcriptional control using a limited number of different transcription factors. In higher eukaryotes, for instance, the superfamily of nuclear hormone receptors and the Fos/Jun and activating

transcription factor/cAMP-responsive-element-binding protein families are well known for this behaviour (Chambon, 1996; Lamb and McKnight, 1991). It was not clear whether this principle was also used in the Zn<sub>2</sub>Cys<sub>6</sub> proteins of *S. cerevisiae*. The well-studied members of this family interact with their DNA-binding sites as homodimers. The Pip2p-Oaf1p heterodimer is an exception to this rule. Indirect evidence suggests that a situation similar to Pip2p and Oaf1p may hold also for the related family members Pdr1p and Pdr3p, which are involved in multi-drug-resistance processes in yeast. The *PDR3* gene is positively autoregulated by its own product by binding to sequences in the 5' region of the gene. Moreover, activation of the *PDR3* gene is dependent on transcription factors Pdr1p and Pdr3p (Delahodde et al., 1995). Our results with Pip2p and Oaf1p show that heterodimer formation between certain members of the Zn<sub>2</sub>Cys<sub>6</sub> family is a possibility. Schjerling and Holmberg (1996) indicated that overexpression was used in many investigations in which direct *in vivo* DNA binding of distinct Zn<sub>2</sub>Cys<sub>6</sub> proteins was assayed. If the situation we described here for Pip2p and Oaf1p applies to more Zn<sub>2</sub>Cys<sub>6</sub> family members, there is a possibility that factors that were described to homodimerise under overexpression conditions may form heterodimers under physiological conditions.

Additional work is needed to see to what extent this principle of combinatorial control occurs in *S. cerevisiae*. A first positive indication was observed in experiments in which we overproduced Pip2p and Oaf1p and used extracts from such cells to study the formation of gel-retarded complexes using ORE from different promoters. The ORE of the *FOX3* gene gave rise to equal amounts of homodimeric Oaf1p and heterodimeric Pip2p-Oaf1p complexes; with the ORE of the *FOX1* gene this ratio was different and much less of the heterodimeric complex was found (Fig. 4). These results might indicate that gene expression is tuned by a combination of the sequence of the ORE and the type of dimeric transcription complex that is bound to it.

Questions that need to be resolved are how the primary signal elicited by the presence of oleate in the growth medium is relayed to the Pip2p · Oaf1p complex and which of the two proteins receives the signal. Further research is needed to distinguish between several types of regulatory mechanisms. One possibility is direct stimulation by low-molecular-mass ligands such as acyl-CoA esters, a concept proposed for the activation of PPAR $\alpha$  in mammals. In *E. coli*, direct binding of acyl-CoA to the transcriptional repressor FadR has been demonstrated, relieving genes encoding fatty acid degradative enzymes from repression (Raman and DiRusso, 1995). A different mechanism could be a relay via a protein-kinase signal-transduction cascade. In this connection the serine/threonine protein kinase Snf1p is somehow involved in the oleate-induction process and in general derepression events (Celenza and Carlson, 1986; Einerhand et al., 1992; Simon et al., 1992). Alternatively, in analogy with Gal80p controlling the action of Gal4p (Johnston et al., 1987; Lue et al., 1987), additional proteins may be involved in mediating the response elicited by the presence of fatty acids.

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## REFERENCES

- Bradford, M. M. (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilising the principle of protein-dye binding, *Anal. Biochem.* **72**, 248–254.
- Celenza, J. L. & Carlson, M. (1986) A yeast gene that is essential for release from glucose repression encodes a protein kinase, *Science* **233**, 1175–1180.
- Chambon, P. (1996) A decade of molecular biology of retinoic acid receptors, *FASEB J.* **10**, 940–954.
- Delahodde, A., Delaveau, T. & Jacq, C. (1995) Positive autoregulation of the yeast transcription factor Pdr3p, which is involved in control of drug resistance, *Mol. Cell. Biol.* **15**, 4043–4051.
- Distel, B., Erdman, R., Gould, S. J., Blobel, G., Crane, D. I., Cregg, J. M., Dodt, G., Fujiki, Y., Goodman, J. M., Just, W. W., Kiel, J. A. K. W., Kunau, W.-H., Lazarow, P. B., Mannaerts, G. P., Moser, H., Osumi, T., Rachubinski, R. A., Roscher, A., Subramani, S., Tabak, H. F., Valle, D., Van der Klei, I., Van Veldhoven, P. P. & Veenhuis, M. (1996) A unified nomenclature for peroxisome biogenesis, *J. Cell Biol.* **135**, 1–3.
- Einerhand, A. W. C., Voorn-Brouwer, T. M., Erdmann, R., Kunau, W.-H. & Tabak, H. F. (1991) Regulation of transcription of the gene coding for peroxisomal 3-oxoacyl-CoA thiolase of *Saccharomyces cerevisiae*, *Eur. J. Biochem.* **200**, 113–122.
- Einerhand, A. W. C., Van der Leij, I., Kos, W. T., Distel, B. & Tabak, H. F. (1992) Transcriptional regulation of genes encoding proteins involved in biogenesis of peroxisomes in *Saccharomyces cerevisiae*, *Cell Biochem. Funct.* **10**, 185–191.
- Einerhand, A. W. C., Kos, W. T., Distel, B. & Tabak, H. F. (1993) Characterization of a transcriptional control element involved in proliferation of peroxisomes in yeast in response to oleate, *Eur. J. Biochem.* **214**, 323–331.
- Erdmann, R. & Blobel, G. (1995) Giant peroxisomes in oleic acid-induced *Saccharomyces cerevisiae* lacking the peroxisomal membrane protein Pmp27p, *J. Cell Biol.* **128**, 509–523.
- Filipits, M., Simon, M. M., Rapatz, W., Hamilton, B. & Ruis, H. (1993) A *Saccharomyces cerevisiae* upstream activating sequence mediates induction of peroxisome proliferation by fatty acids, *Gene (Amst.)* **132**, 49–55.
- Gartner, A., Nasmyth, K. & Ammerer, G. (1992) Signal transduction in *Saccharomyces cerevisiae* requires tyrosine and threonine phosphorylation of *FUS3* and *KSS1*, *Genes Dev.* **6**, 1280–1292.
- Gietz, R. D. & Sugino, A. (1988) New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites, *Gene (Amst.)* **74**, 527–534.
- Gray, W. M. & Fassler, J. S. (1996) Isolation and analysis of the yeast *TEA1* gene, which encodes a zinc cluster Ty enhancer-binding protein, *Mol. Cell. Biol.* **16**, 347–358.
- Hellauer, K., Rochon, M.-H. & Turcotte, B. (1996) A novel DNA binding motif for yeast zinc cluster proteins: the Leu3p and Pdr3p transcriptional activators recognize everted repeats, *Mol. Cell. Biol.* **16**, 6096–6102.
- Hettema, E., Van Roermund, C. W. T., Distel, B., Van den Berg, M., Vilda, C., Rodrigues-Pousada, C., Wanders, R. J. A. & Tabak, H. F. (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.
- Hiltunen, J. K., Wenzel, B., Beyer, A., Erdmann, R., Fossa, A. & Kunau, W. H. (1992) Peroxisomal multifunctional  $\beta$ -oxidation protein of *Saccharomyces cerevisiae*. Molecular analysis of the *FOX2* gene and gene product, *J. Biol. Chem.* **267**, 6646–6653.
- Holmberg, S. & Schjerling, P. (1996) Cha4p of *Saccharomyces cerevisiae* activates transcription via serine/threonine responsive elements, *Genetics* **144**, 467–478.
- Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) Transformation of intact yeast cells treated with alkali cations, *J. Bacteriol.* **153**, 163–168.
- Ji, X., Zhang, P., Armstrong, R. N. & Gilliland, G. L. (1992) The three-dimensional structure of a glutathione S-transferase from the Mu gene class. Structural analysis of the binary complex of isoenzyme 3-3 and glutathione at 2.2-Å resolution, *Biochemistry* **31**, 10169–10184.



- Johnston, S. A., Salmeron, J. M. J. & Dincher, S. S. (1987) Interaction of positive and negative regulatory proteins in the galactose regulon of yeast, *Cell* 50, 143–146.
- Jones, E. W. (1977) Proteinase mutants of *Saccharomyces cerevisiae*, *Genetics* 85, 23–33.
- Jones, S. J. & Prakash, L. (1990) Yeast *Saccharomyces cerevisiae* selectable markers in pUC18 polylinkers, *Yeast* 6, 363–366.
- Karpichev, I. V., Luo, Y., Marians, R. C. & Small, G. M. (1997) A complex containing two transcription factors regulates peroxisome proliferation and the coordinate induction of  $\beta$ -oxidation enzymes in *Saccharomyces cerevisiae*, *Mol. Cell. Biol.* 17, 69–80.
- Lamb, P. & McKnight, S. L. (1991) Diversity and specificity in transcriptional regulation: the benefits of heterotypic dimerization, *Trends Biochem. Sci.* 16, 417–422.
- Lee, S. S.-T., Pineau, J., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salguero, P. M., Westphal, H. & Gonzalez, F. J. (1995) Targeted disruption of the  $\alpha$  isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators, *Mol. Cell. Biol.* 15, 3012–3022.
- Lock, E. A., Mitchell, A. M. & Elcombe, C. R. (1989) Biochemical mechanisms of induction of hepatic peroxisome proliferation, *Annu. Rev. Pharmacol. Toxicol.* 29, 145–163.
- Lue, N. F., Chasman, D. I., Buchman, A. R. & Kornberg, R. D. (1987) Interaction of *GAL4* and *GAL80* gene regulatory proteins *in vitro*, *Mol. Cell. Biol.* 7, 3446–3451.
- Luo, Y., Karpichev, I. V., Kohanski, R. & Small, G. M. (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.
- Marmorstein, R. & Harrison, S. C. (1994) Crystal structure of a PPR1-DNA complex: DNA recognition by proteins containing a Zn<sub>2</sub>Cys<sub>6</sub> binuclear cluster, *Genes Dev.* 8, 2504–2512.
- Myers, A. M., Tzagoloff, A., Kinney, D. M. & Lusty, C. J. (1986) Yeast shuttle and integrative vectors with multiple cloning sites suitable for the construction of *lacZ* fusions, *Gene (Amst.)* 45, 299–310.
- O'Shea, E. K., Rutkowski, R., Stafford, W. F. III & Kim, P. S. (1989) Preferential heterodimer formation by isolated leucine zippers from Fos and Jun, *Science* 245, 646–648.
- O'Shea, E. K., Rutkowski, R. & Kim, P. S. (1992) Mechanism of specificity in the Fos-Jun oncoprotein heterodimer, *Cell* 68, 699–708.
- Piatti, S., Böhm, T., Cocker, J. H., Diffley, J. F. X. & Nasmyth, K. (1996) Activation of S-phase-promoting CDKs in late G1 defines a 'point of no return' after which Cdc6 synthesis cannot promote DNA replication in yeast, *Genes Dev.* 10, 1516–1531.
- Raman, N. & DiRusso, C. C. (1995) Analysis of acyl coenzyme A binding to the transcription factor FadR and identification of amino acid residues in the carboxyl terminus required for ligand binding, *J. Biol. Chem.* 270, 1092–1097.
- Reddy, J. K. & Mannaerts, G. P. (1994) Peroxisomal lipid metabolism, *Annu. Rev. Nutr.* 14, 343–370.
- Reece, R. J. & Ptashne, M. (1993) Determinants of binding-site specificity among yeast C<sub>6</sub> zinc cluster proteins, *Science* 261, 909–911.
- Rottensteiner, H., Kal, A. J., Filipits, M., Binder, M., Hamilton, B., Tabak, H. F. & Ruis, H. (1996) Pip2p: a transcriptional regulator of peroxisome proliferation in the yeast *Saccharomyces cerevisiae*, *EMBO J.* 15, 2924–2934.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular cloning: a laboratory manual*, 2nd edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.
- Schjerling, P. & Holmberg, S. (1996) Comparative amino acid sequence analysis of the C6 zinc cluster family of transcriptional regulators, *Nucleic Acids Res.* 24, 4599–4607.
- Schoonjans, K., Staels, B. & Auwerx, J. (1996) The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation, *Biochim. Biophys. Acta* 1302, 93–109.
- Simon, M., Binder, M., Adam, G., Hartig, A. & Ruis, H. (1992) Control of peroxisome proliferation in *Saccharomyces cerevisiae* by *ADR1*, *SNF1* (*CAT1*, *CCR1*) and *SNF4* (*CAT3*), *Yeast* 8, 303–309.
- Smeal, T., Angel, P., Meek, J. & Karin, M. (1989) Different requirements for formation of Jun:Jun and Jun:Fos complexes, *Genes Dev.* 3, 2091–2100.
- Tyers, M., Tokiwa, G. & Futcher, B. (1993) Comparison of *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins, *EMBO J.* 12, 1955–1968.
- Van den Bosch, H., Schutgens, R. B. H., Wanders, R. J. A. & Tager, J. M. (1992) Biochemistry of peroxisomes, *Annu. Rev. Biochem.* 61, 157–197.
- Veenhuis, M., Mateblowski, M., Kunau, W. H. & Harder, W. (1987) Proliferation of microbodies in *Saccharomyces cerevisiae*, *Yeast* 3, 77–84.
- Wach, A., Brachat, A., Pöhlmann, R. & Philippsen, P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*, *Yeast* 10, 1793–1808.
- Wang, T., Luo, Y. & Small, G. M. (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.
- Wolfe, K. H. & Shields, D. C. (1997) Molecular evidence for an ancient duplication of the entire yeast genome, *Nature* 387, 708–713.
- Zhang, L. & Guarente, L. (1994) The yeast activator *HAP1* – a *GAL4* family member – binds DNA in a directly repeated orientation, *Genes Dev.* 8, 2110–2119.