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The *Candida boidinii* Peroxisomal Membrane Protein Pmp30 Has a Role in Peroxisomal Proliferation and Is Functionally Homologous to Pmp27 from *Saccharomyces cerevisiae*

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The mechanism of peroxisome proliferation is poorly understood. *Candida boidinii* is a methylotrophic yeast that undergoes rapid and massive peroxisome proliferation and serves as a good model system for this process. Pmp30A and Pmp30B (formerly designated Pmp31 and Pmp32, respectively) are two closely related proteins in a polyploid strain of this yeast that are strongly induced by diverse peroxisome proliferators such as methanol, oleate, and D-alanine. The function of these proteins is not understood. To study this issue, we used a recently described haploid strain (S2) of *C. boidinii* that can be manipulated genetically. We now report that strain S2 contains a single *PMP30* gene very similar in sequence (greater than 93% identity at the DNA level) to *PMP30A* and *PMP30B*. When *PMP30* was disrupted, cell growth on methanol was greatly inhibited, and cells grown in both methanol and oleate had fewer, larger, and more spherical peroxisomes than wild-type cells. A similar phenotype was recently described for *Saccharomyces cerevisiae* cultured on oleate in which *PMP27*, which encodes a protein of related sequence that is important for peroxisome proliferation, was disrupted. To determine whether Pmp27 is a functional homolog of Pmp30, genetic complementation was performed. *PMP30A* was expressed in the *PMP27* disruptant of *S. cerevisiae*, and *PMP27* was expressed in the *PMP30* disruptant of *C. boidinii* S2. Complementation, in terms of both cell growth and organelle size, shape, and number, was successful in both directions, although reversion to a wild-type phenotype was only partial for the *PMP30* disruptant. We conclude that these proteins are functional homologs and that both Pmp30 and Pmp27 have a direct role in proliferation and organelle size rather than a role in a specific peroxisomal metabolic pathway of substrate utilization.

Peroxisomes are organelles bound by a single membrane that perform diverse metabolic functions, many of which are oxidative in nature. The importance of peroxisomes has been well illustrated by several human peroxisomal disorders. The most severe of them, Zellweger syndrome and neonatal adrenoleukodystrophy, cause profound defects in protein import (24).

While our knowledge of peroxisomal targeting has increased greatly in the past several years, the mechanisms of peroxisome division and assembly are not well understood. Several fungal model systems are useful for the study of these processes, since massive proliferation in these cells can be induced by growth substrates (1).

In the methylotrophic yeast *Candida boidinii*, peroxisomes can be induced to proliferate by diverse carbon sources, among them oleate, D-alanine, and methanol (25). These organelles fill up with the enzymes required to assimilate the specific growth substrates (9, 17). However, the morphology of these proliferated organelles is different. Cells fully induced by methanol typically contain a cluster of adherent large peroxisomes, probably derived from a single small organelle (27). In contrast, oleate-induced cells have smaller, more numerous peroxisomes that are separated and distributed throughout the cytoplasm (9).

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Unlike most peroxisomal matrix proteins, which are induced in response to specific growth substrates, there are abundant proteins of the peroxisomal membrane that are induced by all of the proliferating substrates. Two of these are Pmp31 and Pmp32 from a polyploid strain of *C. boidinii* (9). Since the corresponding genes are probably allelic (see below) and the molecular masses of the proteins are closer to 30 kDa (13), we propose to rename the genes *PMP30A* and *PMP30B* and the corresponding proteins Pmp30A and Pmp30B (formerly Pmp31 and Pmp32, respectively). Their functions are unknown. However, they both have 37% sequence identity with a recently identified protein of *Saccharomyces cerevisiae*, Pmp27 (6, 11). *S. cerevisiae* undergoes peroxisome proliferation only in response to fatty acids such as oleate (28). In the absence of Pmp27, a few large peroxisomes are generated instead of many smaller ones, as if peroxisomal fission were inhibited. Furthermore, overproduction of Pmp27 results in a much higher degree of organelle proliferation, so that many more peroxisomes are observed than in the wild type. These data suggest that Pmp27 is important, although not absolutely essential, for peroxisome proliferation. However, another possibility is that the aberrancies in organellar morphology and number that are caused by manipulating the levels of Pmp27 are an indirect effect of a role of this protein in oleate metabolism. This view is strengthened by the appearance of large peroxisomes in animal cells in which β-oxidation is defective (14).

We have recently identified a haploid strain of *C. boidinii* (S2) and have developed the molecular tools to allow gene disruption and expression of foreign genes in this yeast (15, 16,

19). We now report that this strain has only one *PMP30* gene. A strain with *PMP30* disrupted grows very poorly on methanol and contains large and aberrant peroxisomes. Furthermore, we show in genetic complementation experiments that *PMP27* and *PMP30* are functional homologs. These data strongly support the hypothesis that these proteins directly promote peroxisome proliferation and affect organelle size and shape.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* JM109, JM110, and TG1 (20) were used for plasmid propagation and for the construction of the *C. boidinii* S2 genomic library. pBluescript II SK$^+$ and pBluescript KS$^-$ were from Stratagene Ltd. (La Jolla, Calif.), and pUC18 was from New England Biolabs (Beverly, Mass.).

DNA and RNA methods. Yeast DNA was purified by the method of Cryer et al. (4) or Davis et al. (5). Southern analysis was performed as described previously (16). [α-^{32}P]dCTP was purchased from Amersham Corp. (Arlington Heights, Ill.). Transformation of *S. cerevisiae* and *C. boidinii* TK62 was performed by the conventional (10) and modified lithium acetate (15) methods, respectively.

Yeast strains, media, and cultivation. (i) Cultivation of *C. boidinii*. *C. boidinii* TK62 (*ura3*), which was isolated from *C. boidinii* S2 in a previous study (16), was used as the host for transformation. *C. boidinii* transformant strain GC (gene convertant) (18) was used as the wild-type control strain. Cells were cultured in basal MI medium as described previously (18). The concentrations of the carbon sources were 0.7% (vol/vol) methanol, 0.5% (vol/vol) oleate, 0.6% (wt/vol) D-alanine, 2% (wt/vol) potassium acetate, 3% (wt/vol) glycerol, or 2% (wt/vol) glucose; Tween 80 (Sigma) was added to the oleate medium at 0.05% (vol/vol). The initial pH of the medium was adjusted to 6.0. Cultivation was performed in a shaker incubator at 28°C, and growth was monitored by measuring light scattering at 610 nm. For cultures with oleate, measurements of cell density were blanked with the same medium without inoculum, diluted appropriately.

(ii) Cultivation of *S. cerevisiae*. All experiments with *S. cerevisiae* utilized the *Pmp27* disruptant strain 3B (11). This strain was transformed with plasmids containing *PMP27*, *PMP30A*, or no insert, as indicated. Culturing for the determination of growth characteristics or other purposes was performed as previously described (11).

Electron microscopy. *C. boidinii* was grown on glucose medium containing 0.5% yeast extract, inoculated into synthetic oleate or methanol medium at an optical density at 610 nm (OD_{610}) of 0.1 for the wild-type strain and 1.0 for strain D30BP, and cultivated for 10 h. Cells were washed three times with H_2O, suspended in 100 mM potassium phosphate buffer (pH 7.5), and fixed by adding 25% (wt/vol) glutaraldehyde to a final concentration of 2.0% (wt/vol). After standing for 2 h at 4°C, the cells were washed with the buffer and postfixed sequentially with 1.5% (wt/vol) $KMnO_4$ and 1.5% (wt/vol) uranyl acetate. The material was dehydrated in a graded acetone series and then embedded in Spurr resin (Polysciences, Inc., Warrington, Pa.). Ultrathin sections were prepared by using a diamond knife for cutting and observed under an electron microscope (JEOL model C100).

S. cerevisiae was precultured in SGd (yeast nitrogen base containing 3% glycerol and 0.1% glucose) and then cultured in oleate medium for 16 h, as described previously (12). Cells were collected by centrifugation and fixed in 200 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH ca. 6.7] containing 4% paraformaldehyde and 0.5% glutaraldehyde for 2 h at room temperature. The cell pellet was infused with 2 M sucrose containing 3% polyvinylpyrrolidone (molecular weight, 10,000; Sigma) for 2 h. Samples were frozen, sectioned, and prepared for immunoelectron microscopy as previously described (12), using a 1:500 dilution of antithiolase antibody (gift from Jon Rothblatt, Dartmouth College). They were viewed on a JEOL model 1200 electron microscope.

Isolation of *PMP30* from *C. boidinii* S2 and gene disruption. (i) Cloning and sequence. Two nonoverlapping probes derived from *PMP30A* of *C. boidinii* ATCC 32195 were used to clone the *PMP30* gene: a 1.9-kbp *Hin*dIII-*Sal*I fragment and a 1.3-kbp *Hin*dIII-*Pst*I fragment (13). These inserts were gel purified and labeled with [α-^{32}P]dCTP by the method of Feinberg and Vogelstein (7). Southern analysis of *C. boidinii* S2 genomic DNA digested with *Xba*I and probed with either *PMP30A* fragment revealed a single 4.8-kbp band. A pool of *Xba*I-digested genomic DNA of approximately this size was gel purified and ligated into the *Xba*I site of pBluescript II SK$^+$. Transformants were transferred onto a Biodyne nylon membrane (Pall Bio Support, New York, N.Y.). After lysis of the bacteria and binding of the liberated DNA to the nylon membrane, these blots were used for colony hybridization under high-stringency hybridization conditions with Church-Gilbert buffer (1% bovine serum albumin, 1 mM EDTA, 0.25 M NaCl, 0.25 M $NaPO_4$ [pH 7.2], 7% sodium dodecyl sulfate [SDS]) (3). Hybridization was performed overnight at 65°C, and then the membranes were washed three times in 0.3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at the same temperature. Four clones that showed strong positive signals were found to harbor a reactive 4.8-kbp *Xba*I fragment. DNA was sequenced by using a PRISM DyeDeoxy Terminator Cycle sequencing kit and a DNA sequencer (model 373A; Applied Biosystems) or as described previously (19). All four clones contained the *C. boidinii* PMP30 gene.

(ii) Gene disruption. The 2.8-kbp *Xba*I-*Hin*dIII fragment of pMP301 (Fig. 1B) harboring *C. boidinii PMP30* was subcloned into pBluescript II SK$^+$, yielding pMP302. This plasmid was digested with *Nde*I and *Spe*I to delete a 0.9-kbp fragment which included most of the *C. boidinii PMP30* gene. The remaining fragment was gel purified, blunted with T4 DNA polymerase, and ligated to each of the following in separate reactions after being treated with T4 polymerase: the *Bam*HI-*Pst*I fragment of *C. boidinii URA3* DNA, and the insert from pLSP (*C. boidinii URA3* gene having repeated sequences at the 5′- and 3′-flanking regions) (19). The ligation reactions generated the *C. boidinii PMP30* disruption vectors pMP30BP and pMP30SBR, respectively (Fig. 1B). After propagation of pMP30BP and pMP30SBR in *E. coli*, both insert DNA fragments were isolated following *Hin*dIII-*Sac*I double digestion, and these fragments were used to transform *C. boidinii* TK62 to uracil prototrophy. The disruption of *PMP30* was confirmed by genomic Southern analysis of *Bgl*II-digested DNA from a Ura$^+$ transformant, using the 2.8-kbp *Xba*I-*Eco*RV fragment from pMP302 as the probe. The *PMP30* disruptant strain D30SBR, obtained with pMP30SBR, was reverted to uracil auxotrophy, producing strain D30U, by our previously described procedure (19).

Introduction of *S. cerevisiae PMP27* into *C. boidinii*. The construction of the universal vector pNoteI for expression in *C. boidinii* will be described elsewhere. pNoteI harbored the *C. boidinii AOD1* promoter and terminator sequences and the *C. boidinii URA3* gene as the selectable marker, with a unique *Not*I site to insert coding sequences for expression. *Not*I sites were added at both ends of the *S. cerevisiae PMP27* coding sequence by PCR. The two primers were 27N (ATA AGAATGCGGCCGCAATATGGTCTGTGATACACTGG) and 27R (ATAA GAATGCGGCCGCCTATGTAGCTTTCCACATGTCTTG), and *S. cerevisiae PMP27* DNA served as the template in the reaction. The amplified fragment was purified from an agarose gel, digested with *Not*I, and inserted into pNoteI, yielding pExp27. The proper orientation of the inserted fragment was confirmed by physical mapping. The plasmid was linearized with *Bam*HI and introduced into strain D30U.

Introduction of *C. boidinii PMP30A* into *S. cerevisiae*. Plasmid 31-9C, containing *PMP30A* (13), was linearized with *Acc*I, blunted with Klenow, and digested with *Nhe*I. The resulting fragment, which contained the entire *PMP30A* coding region flanked by 11 bp upstream of the initiator ATG and 2 bp downstream of the stop codon, was ligated into the *Sma*I and *Xba*I sites of pUC18. The insert was then removed from this construct by digestion in the polylinker region with *Eco*RI and *Pst*I. This fragment was ligated into the corresponding sites in pKS27poly. This vector, which will fully be described elsewhere (5a), consists of *PMP27* cloned into pBluescript KS$^-$ in which the coding region for Pmp27 was replaced by a polylinker. The ligation placed the *PMP30A* coding region behind the *PMP27* promoter and in front of the *PMP27* terminator. A fragment with all of these elements was liberated with *Cla*I and *Bam*HI and ligated into pRS313 (*CEN6 HIS3*) (22), to produce pRSPMP30, or liberated with *Kpn*I and *Bam*HI and ligated into YEplac112 (2μm *TRP1*) (8).

Other methods. Immunoblots were performed as described by Towbin et al. (26), using the previously described antibodies against *S. cerevisiae* Pmp27 and *C. boidinii* Pmp30 (11, 13).

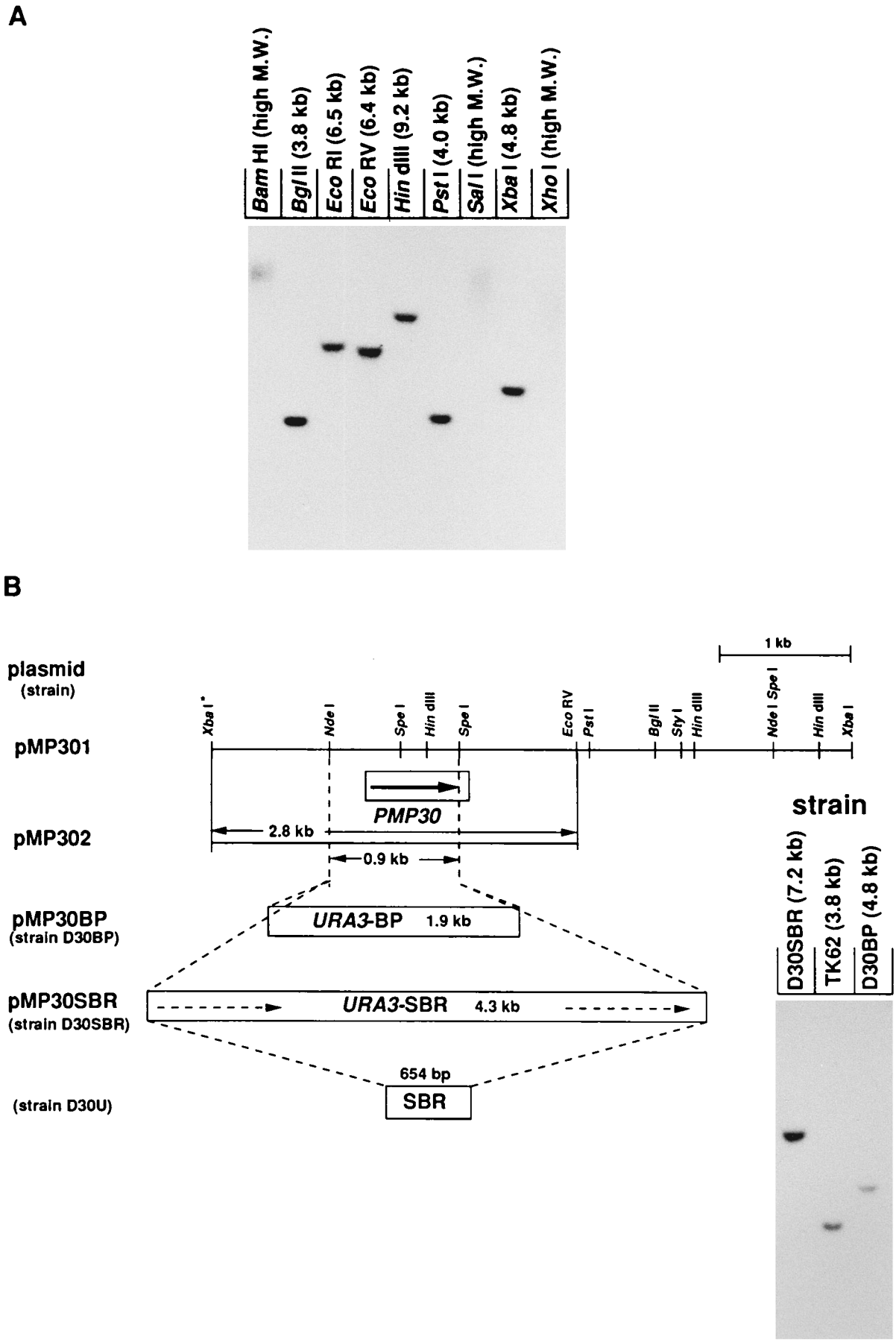
Nucleotide sequence accession number. The *PMP30* sequence has been assigned GenBank accession number U36243.

RESULTS

Pmp30 encoded by a single gene in *C. boidinii* S2. In a previous study, two closely related genes, termed *PMP30A* (*PMP31*) and *PMP30B* (*PMP32*), were isolated from *C. boidinii* ATCC 32195, a polyploid strain (13). To determine if a haploid strain of *C. boidinii* S2 also contained two *PMP30* genes, genomic Southern analysis of *C. boidinii* S2 DNA was performed with radiolabeled *PMP30A* or *PMP30B* as the probe. Only one fragment from each digestion bound to the *PMP30A* probe (Fig. 1A). Identical results were seen with the *PMP30B* probe (data not shown). These results and the gene disruption experiments described below indicate that *C. boidinii* S2 contains only one gene for Pmp30, which we name *PMP30*.

PMP30 was isolated by colony hybridization, using radiolabeled *PMP30A* as the probe. Restriction mapping and se-

FIG. 1. (A) Southern analysis of genomic DNA from *C. boidinii* S2 probed with ^{32}P-labeled *PMP30A* DNA from *C. boidinii* ATCC 32195. The sizes of hybridizing bands are shown in parentheses in each lane. M.W., molecular weight. (B) Physical map of the cloned *PMP30* gene, the disruption strategy, and genomic Southern analysis. *Bgl*II-digested total DNA from two disruptant strains, D30SBR and D30BP, and the host strain TK62 was probed with ^{32}P-labeled pMP302 insert DNA. The names of plasmids used for gene disruption together with the derived strains are also shown at the left. *URA3*-BP and *URA3*-SBR represent the 1.9-kb *Bam*HI-*Pst*I fragment from the *C. boidinii URA3* gene (16) and the pSBR insert DNA (19), respectively.


A
Bam HI (high M.W.)
Bgl II (3.8 kb)
Eco RI (6.5 kb)
Eco RV (6.4 kb)
Hin dIII (9.2 kb)
Pst I (4.0 kb)
Sal I (high M.W.)
Xba I (4.8 kb)
Xho I (high M.W.)
B
plasmid
(strain)
1 kb
Xba I*
Nde I
Spe I
Hin dIII
Spe I
Eco RV
Pst I
Bgl II
Sty I
Hin dIII
Nde I Spe I
Hin dIII
Xba I
pMP301
PMP30
pMP302
2.8 kb
0.9 kb
pMP30BP
(strain D30BP)
URA3-BP 1.9 kb
pMP30SBR
(strain D30SBR)
URA3-SBR 4.3 kb
654 bp
SBR
(strain D30U)
strain
D30SBR (7.2 kb)
TK62 (3.8 kb)
D30BP (4.8 kb)

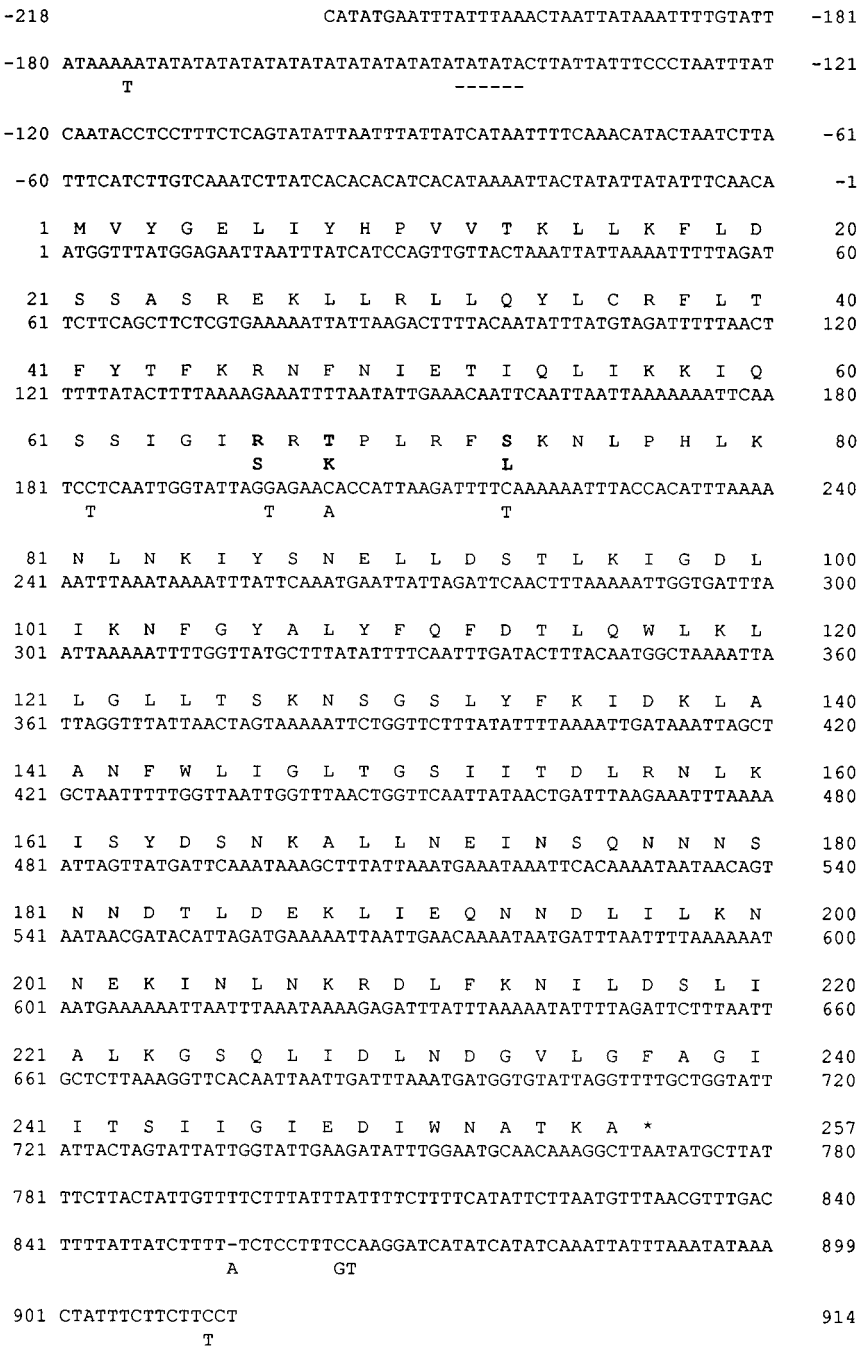


FIG. 2. Nucleotide sequence and deduced amino acid sequence of *PMP30* from *C. boidinii* S2 and comparison with *PMP30B* from *C. boidinii* ATCC 32195. Upper sequences represent the *PMP30* gene. Differences in amino acid sequence are shown in boldface type. Dashes signify deleted bases or amino acids.

quence analysis showed nearly perfect identity to *PMP30B* (Fig. 2). DNA within the coding region was 99% identical to *PMP30B*, differing at only three codons. It was also 93% identical to *PMP30A* (not shown). The sequenced portions of 5′ and 3′ untranslated regions were also highly conserved. We conclude that *PMP30A* (*PMP31*) and *PMP30B* (*PMP32*) are probably allelic in the polyploid *C. boidinii* strain and that the Pmp30s probably perform the same function.

Next, two *PMP30* disruption vectors were constructed (Fig. 1B). In pMP30BP, the coding region and flanking 5′ untranslated sequences of *PMP30* were replaced with *URA3* of *C. boidinii* (19). The other plasmid, pMP30SBR, also contained repeated sequences upstream and downstream of *URA3* (dashed arrows in the figure), enabling later regeneration of uracil auxotrophy after *PMP30* disruption (19). *C. boidinii* TK62, the *ura3* derivative of strain S2 (16), was transformed with pMP30BP and pMP30SBR, generating strains D30BP and D30SBR, respectively. Disruption of *PMP30* was confirmed by Southern analysis with *Bgl*II-digested DNA from each transformant. The DNA from the host strain gave a single band of 3.8 kbp. It shifted to 4.8 kbp in strain D30BP and to 7.2 kbp in strain D30SBR, as expected for a disruption caused by homologous recombination. In addition, Western blotting with an anti-Pmp30 antibody revealed loss of signal in the two

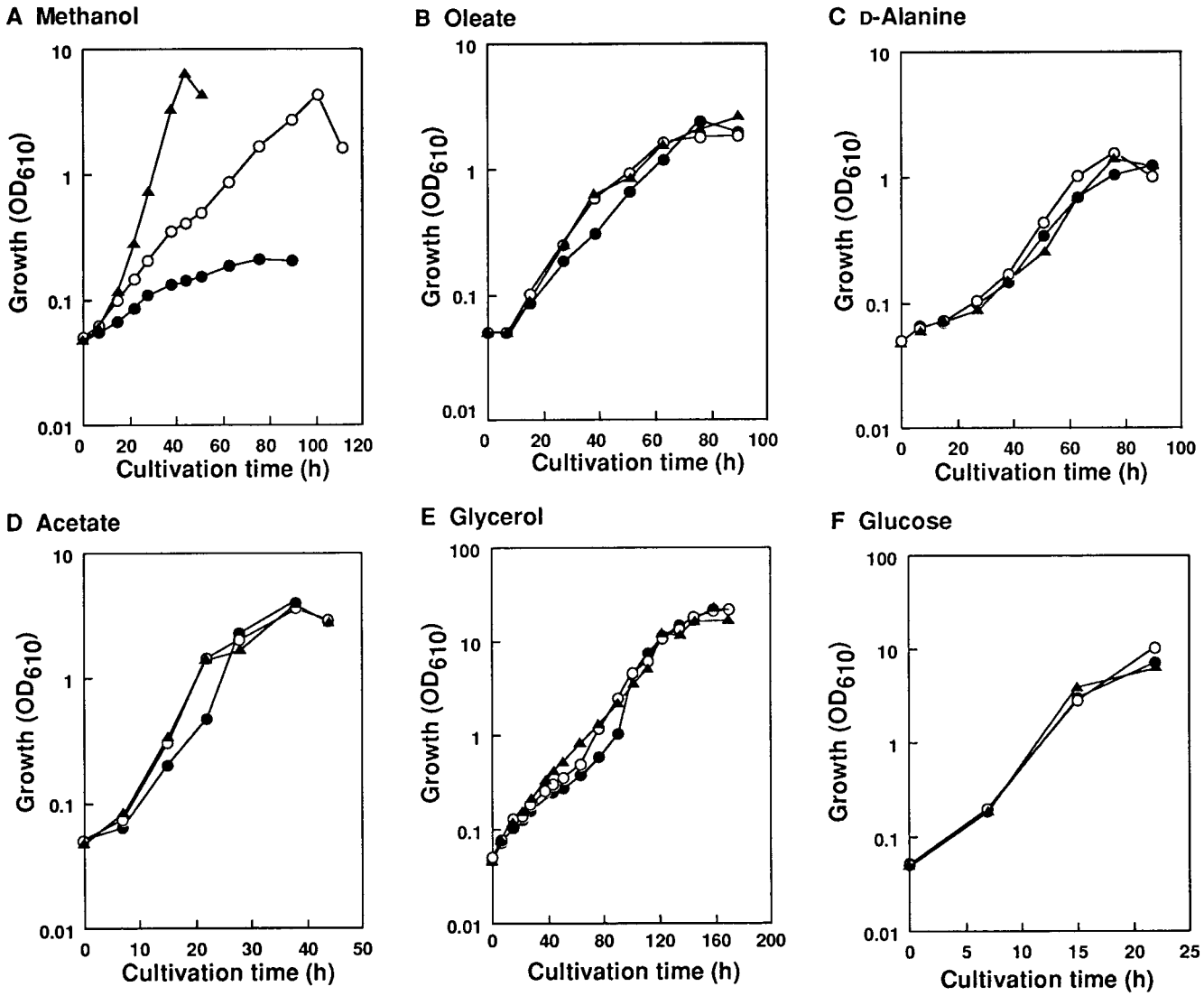


FIG. 3. *PMP27* restores the growth of the *PMP30* disruptant to various extents. The growth kinetics of *C. boidinii* D30BP (*PMP30* disruptant, ●), the wild-type strain (▲), and strain N27D30 (D30BP expressing *PMP27*, ○), were determined. Carbon sources are indicated.

disruptant strains (not shown). These results confirmed that the haploid strain of *C. boidinii* contained only one *PMP30* gene.

Growth analysis. Mutants of *C. boidinii* that are severely deficient in peroxisome function can be identified because they are unable to grow on oleate, D-alanine, or methanol as the sole carbon source (17). In contrast, we found that the *PMP30* disruption strain D30BP was able to grow on plates containing these carbon sources. This result suggested that functional peroxisomes were present in the absence of Pmp30. We compared the growth rates in liquid medium of the wild-type strain and strain D30BP in several growth substrates (Fig. 3). There was no effect of the *PMP30* disruption on growth in glucose and very little effect on growth in D-alanine (D-alanine was used as a single carbon and nitrogen source). In contrast, the disruption caused a small but reproducible inhibition (in three experiments) of growth in oleate, acetate, and glycerol and a dramatic inhibition of growth in methanol. Thus, the effects of growth in "peroxisomal substrates" varied from almost none (in D-alanine) to severe (in methanol).

Effect of disruption of *PMP30* on peroxisome morphology. The subcellular morphology of *C. boidinii* D30BP and the corresponding wild-type strain was compared. Cells were cultured in methanol- or oleate-containing medium for 10 h, at which time peroxisome proliferation reaches a steady state (27). Cells were then fixed with glutaraldehyde-$KMnO_4$. When the wild-type strain was grown in methanol, a spheroid of about 2 μm in diameter, composed of three or four tightly packed peroxisomes, was present in each cell, suggesting that the peroxisomes had originated from a single progenitor organelle (Fig. 4A). In contrast, the peroxisomes of the methanol-induced D30BP strain were characterized by the existence of one or two unusually spherical peroxisomes of ca. 0.7 to 1.7 μm, although we also sometimes observed cells which also contained peroxisomes of normal size (Fig. 4B). The large round methanol-induced peroxisomes, which have not been observed previously in *C. boidinii* S2, suggested that segmentation of the original peroxisome had not occurred, while import of matrix proteins proceeded as normal.

A similar but more dramatic effect was observed with oleate-induced cells. Wild-type cells had ca. 5 to 12 small peroxisomes of 0.1 to 0.7 μm (Fig. 4C). In contrast, oleate-induced cells

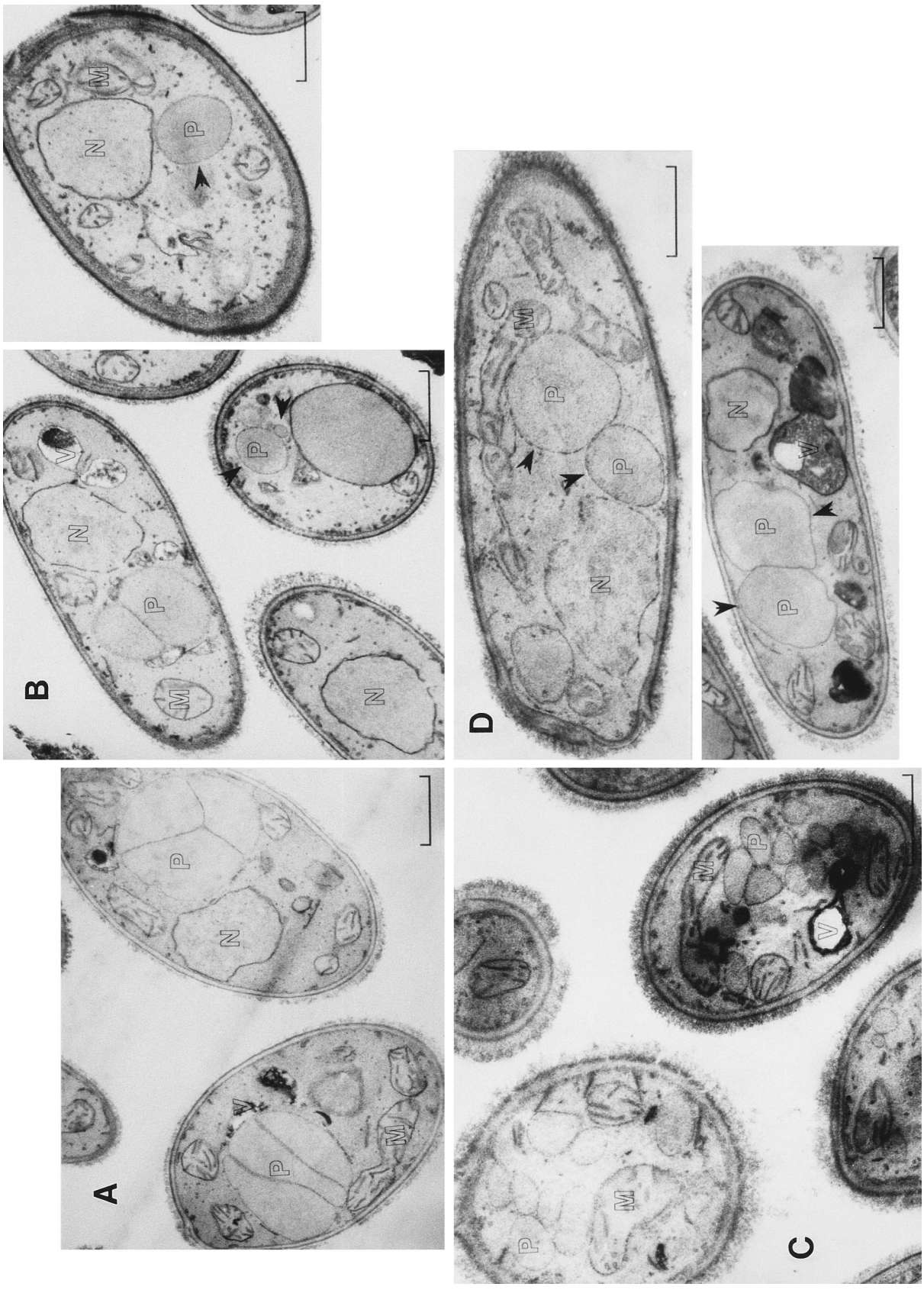


FIG. 4. Large and irregular peroxisomes in the *PMP30* disruptant. (A and B) Methanol-induced cells of *C. boidinii* wild-type strain (A) and strain D30BP (B); (C and D) oleic acid-induced cells of *C. boidinii* wild-type strain (C) and strain D30BP (D). Arrowheads indicate round peroxisomes observed in strain D30BP. P, peroxisome; N, nucleus; M, mitochondrion; V, vacuole. Bars, 1 μm.

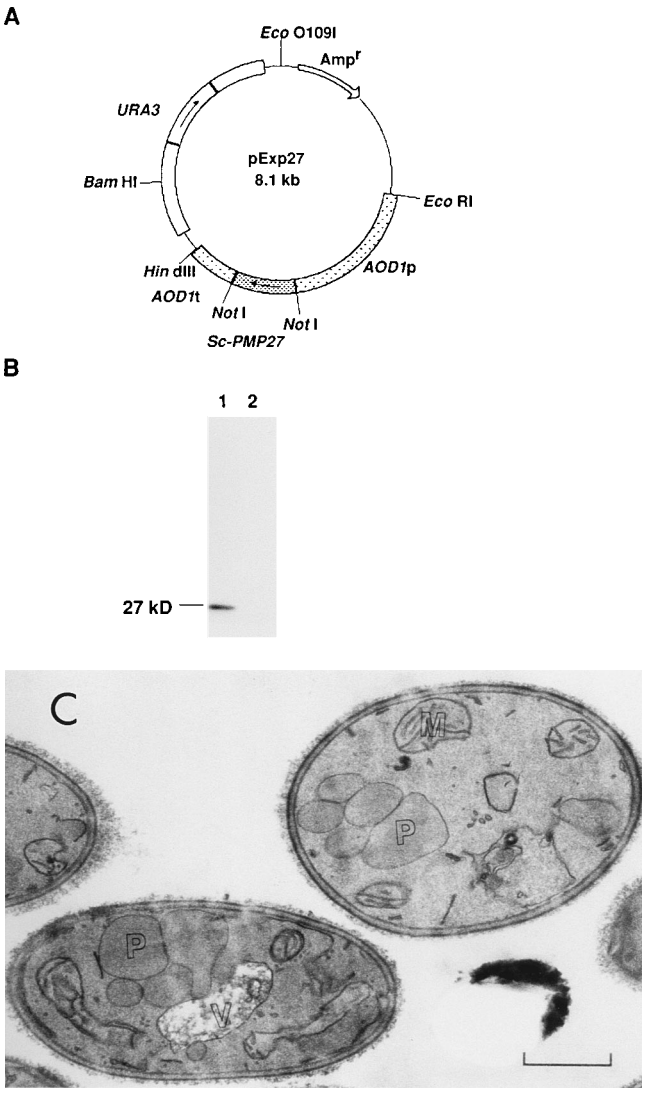


FIG. 5. Expression of *S. cerevisiae PMP27* in the *C. boidinii PMP30* disruptant. (A) Plasmid used for expression of *PMP27*. (B) Immunoblot analysis. Lane 1, wild type; lane 2, *C. boidinii* N27D30. (C) Peroxisome morphology of methanol-induced cells of *C. boidinii* N27D30. Symbols are the same as in Fig. 4. Bar, 1 μm.

of strain D30BP had only one or two giant peroxisomes (Fig. 4D).

***PMP27* can partially complement the *PMP30* disruption strain.** Since this observed phenotype was very similar to that of the *PMP27* disruption strain of *S. cerevisiae* (6, 11) and the sequence of *S. cerevisiae* Pmp27 is about 35% identical to that of Pmp30 of *C. boidinii*, we hypothesized that they are functional homologs. To test for functional homology, we first had to convert strain D30SBR to uracil auxotrophy. For this purpose, cells of this strain were treated with 5-fluoro-orotic acid. One spontaneous 5-fluoro-orotic acid-resistant colony was isolated from strain D30SBR and named strain D30U. We assume that *URA3* was lost by virtue of the repeated sequences on both sides of the gene (19). This event was confirmed by Southern analysis, i.e., the 7.2-kbp band of strain SBR shifted to 3.5 kbp in strain D30U (data not shown).

The *S. cerevisiae PMP27* gene was placed under the *C. boidinii AOD1* promoter for overexpression in methanol medium (Fig. 5A) and introduced into *C. boidinii* D30U. Transformation was confirmed by Southern analysis (data not shown). Immunoblot analysis of the transformant strain N27D30 grown on methanol gave a strong signal at 27 kDa, while the control strain did not (Fig. 5B), confirming that Pmp27 was expressed in the *PMP30* disruptant.

As shown in Fig. 3, strain N27D30 showed a much higher growth rate and yield than strain D30BP in methanol-containing medium, indicating that the *PMP30* disruption was partially restored by the introduction of *S. cerevisiae PMP27*.

Next, methanol-induced cells of strain N27D30 were observed by electron microscopy. As shown in Fig. 5C, expression of Pmp27, driven by the strong *AOD1* promoter, produced about 5 to 12 small peroxisomes (0.05 to 1.0 μm) per cell. Therefore, overproduction of Pmp27, which promotes peroxisome proliferation in oleate-induced cells of *S. cerevisiae* (6, 11), also promoted proliferation of methanol-induced peroxisomes in *C. boidinii*. Interestingly, the shapes of the peroxisome clusters in the overproducer resembled those of wild-type cultures grown in oleate more than those from methanol-grown cultures.

Although the observed growth defects of the original *PMP30* disruption strain on glycerol-, acetate-, and oleate-containing media were minor, the introduction of *S. cerevisiae PMP27* restored the growth to nearly wild-type levels. This may be due to a basal level of expression from the *C. boidinii AOD1* promoter in these carbon sources, which has previously been detected with an *AOD1* reporter construct (data not shown). Therefore, *PMP27* was able to partially revert the *PMP30* disruption in terms of both growth and organelle morphology.

***PMP30A* can complement the *PMP27* disruption strain.** Disruption of *PMP27* in *S. cerevisiae* caused slower growth and lower cell yields on glycerol and oleate (11). There was also a slight growth inhibition on acetate medium. (*S. cerevisiae* is unable to grow on methanol.) The *PMP27* disruption strain contained much larger and fewer peroxisomes per cell.

We introduced *C. boidinii PMP30A*, driven by the *PMP27* promoter, into the *S. cerevisiae PMP27* disruption strain 3B. Expression from a low-copy plasmid restored growth on all carbon sources to levels very similar to those with the parallel plasmid with the *PMP27* gene (Fig. 6). Expression of *PMP30A* and *PMP27* from a high-copy plasmid had a toxic effect on growth on both glycerol and oleate (data not shown).

C. boidinii PMP30A also reverted the peroxisome morphology to normal. Figure 7A shows a cell of the *PMP27* disruption strain, with characteristic large and irregular peroxisomes as seen previously (6, 11). Introduction of *PMP27* or *PMP30A* permitted cells to proliferate peroxisomes of normal size and number (Fig. 7B and C, respectively).

We conclude from the double complementation experiments that Pmp27 of *S. cerevisiae* is a homolog of the *C. boidinii* Pmp30 and that these proteins are important for peroxisome proliferation and maintenance of peroxisome shape and size.

DISCUSSION

We have shown that Pmp30 and Pmp27 are functional homologs. Since *S. cerevisiae* cannot utilize methanol, it is striking that Pmp27 can substitute for a protein that is important, albeit indirectly, for methanol utilization in *C. boidinii*. This is the first report of a yeast gene involved with peroxisome assembly exhibiting complementation in a heterologous yeast species. The functional interchangeability between Pmp27 and Pmp30 strongly indicates that they are not metabolism- or organism-specific molecules but instead are universal molecules required for the maintenance of normal peroxisome size and number.

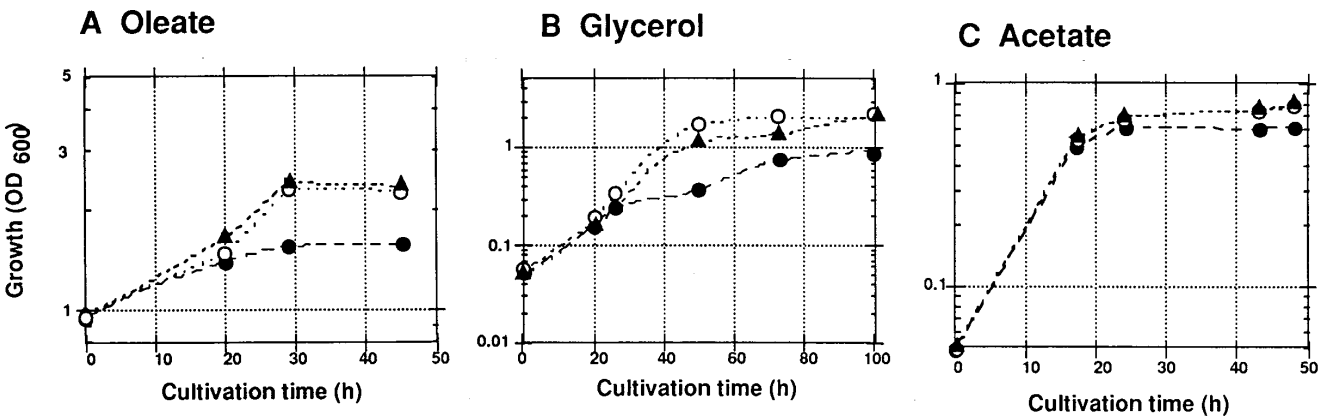


FIG. 6. *PMP30A* restores the growth of the *PMP27* disruptant. Growth of the *PMP27* disruption strain 3B containing various plasmids on the indicated carbon sources was monitored. ●, control plasmid pRS313; ○, pRSPMP31; ▲, pRS27. Dotted line shows the growth of each strain.

The molecular machinery for this process should be conserved, and we expect that a mammalian member of this family will soon be found.

What causes the inhibition of growth in the absence of the Pmp30 family of proteins on selective peroxisome-proliferating carbon sources, methanol for *C. boidinii* and oleate for *S. cerevisiae*? Erdmann and Blobel (6) showed evidence that segregation of peroxisomes between mother and daughter cells is inhibited in the *PMP27* disruption strain, perhaps because of the large size of the organelles. This may explain our current data. Why is the growth of the *PMP30* disruption strain more inhibited on methanol than on oleate, even though the peroxisomes are similar sizes? Wild-type cells in methanol double more than twice as fast as in oleate (Fig. 3). Perhaps the longer cell cycle in oleate medium better accommodates the segregation of the mutant peroxisomes. However, we cannot rule out the possibility that differential exchanges of metabolites between peroxisomal matrix and cytoplasm in the wild-type and disruption strains are responsible for the growth defects that we see.

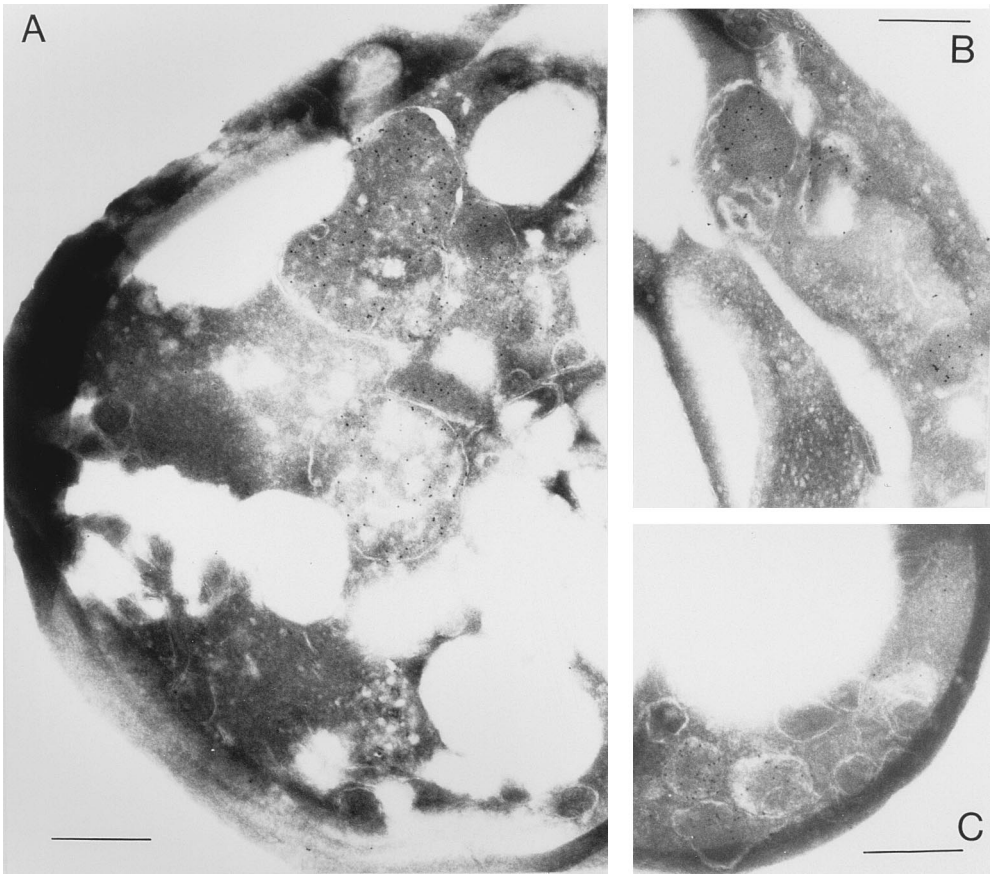


FIG. 7. *PMP30A* complements the peroxisome morphology defect in the *PMP27* disruptant strain. Cells were cultured in oleate for 20 h and then processed for immunoelectron microscopy with antibodies to thiolase. (A) Strain 3B expressing the control plasmid pRS313; (B) 3B expressing pRS27; (C) 3B expressing pRSPMP31. Bars, 500 nm.

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Mdm10p (219-265)  SKFNTSLYNNSSLSLGAEFWLGLVSLSPGCSTTLRYYT
                  ::   :::      :.:.::  :..:. .  : ::
Pmp30  (126-169)  SKNSGSLYFKID-KLAANFW--LIGLTGSIITDLRNLK
                  .   ::  . .   :  :.:: ...  ::: .  : .
Pmp27p (119-162)  VTVLTGK--KIP-RWSNWCW--LFGLLSGLAMDLRKIQ


HSTNTGRPLSKFNTSLYNNSSLSLGAEFWLGLVSLSPGCSTTLRYYTHSTNTGRPL
 : .. ..:::   :::      :.:.::  :..:. .  : ::    : .. ..:
ISYDSNKALSKNSGSLYFKID-KLAANFW--LIGLTGSIITDLRNLKISYDSNKAL
  :.      .   ::  . ..  :  :.:: ...  ::: .  :     :.
TSHAQIAAFVTVLTGK--KIP-RWSNWCW--LFGLLSGLAMDLRKIQTSHAQIAAF
```

FIG. 8. Alignment of the region displaying sequence similarity in the Pmp30 family and *S. cerevisiae* Mdm10p. Colons represent identical residues, and dots represent similar residues. Similar residues: G, A, and S; P, A, and S; S, T, and A; D, E, Q, and N; K and R; V, I, L, M, and F; and F and Y.

Overproduction of some membrane proteins can cause the proliferation of the organelle where it is localized. For example, overproduction of *Candida maltosa* cytochrome P450 in *S. cerevisiae* induced a dramatic proliferation of endoplasmic reticulum even though the heterologous P450 protein was not functional in *S. cerevisiae* (21). It is believed that the membrane proliferation caused by overexpressing cytochrome P450 is a secondary effect of the protein, not directly related to its function. Is the peroxisome proliferation caused by overexpressing Pmp27 (11) also an artifactual effect of this protein? The strongest evidence for a direct effect of the Pmp30 family of proteins on proliferation is the morphology of the organelle in its absence: in both *C. boidinii* and *S. cerevisiae*, we find fewer and much larger peroxisomes. Furthermore, the growth defects found in the disruptions are not carbon source specific; the defect was most pronounced in *C. boidinii* growing on methanol and *S. cerevisiae* growing on oleate, substrates that are metabolized very differently. Taken together, our data indicate that proteins in the Pmp30 family directly promote peroxisome proliferation.

Recently, two mitochondrial outer membrane proteins, *S. cerevisiae* Mdm10p and Mmm1p, were reported to affect mitochondrial size and morphology (2, 23). Disruption of these genes singly led to the generation of giant spherical mitochondria, similar to the peroxisomes in *C. boidinii* D30BP. Furthermore, segments of *S. cerevisiae MDM10* and *C. boidinii PMP30* have considerable homology (Fig. 8). We also observed some homology between *PMP27* and *MMM1* (11). Perhaps some common molecular mechanism exists to maintain organelle shape and number in both mitochondria and peroxisomes.

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