Pex11p Plays a Primary Role in Medium-Chain Fatty Acid Oxidation, a Process that Affects Peroxisome Number and Size in Saccharomyces cerevisiae

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Abstract. The Saccharomyces cerevisiae peroxisomal membrane protein Pex11p has previously been implicated in peroxisome proliferation based on morphological observations of PEX11 mutant cells. Pex11p-deficient cells fail to increase peroxisome number in response to growth on fatty acids and instead accumulate a few giant peroxisomes. We report that mutants deficient in genes required for medium-chain fatty acid (M CFA) β-oxidation display the same phenotype as Pex11p-deficient cells. Upon closer inspection, we found that Pex11p is required for M CFA β-oxidation. Disruption of the PEX11 gene results in impaired formation of M CFA-CoA esters as measured in intact cells, whereas their formation is normal in cell lysates. The sole S. cerevisiae M CFA-CoA synthetase (Faa2p) remains properly localized to the inner leaflet of the peroxisomal membrane in PEX11 mutant cells. Therefore, the in vivo latency of M CFA activation observed in Pex11p-deficient cells suggests that Pex11p provides Faa2p with substrate. When PEX11 mutant cells are shifted from glucose to oleate-containing medium, we observed an immediate deficiency in β-oxidation of M CFA’s whereas giant peroxisomes and a failure to increase peroxisome abundance only became apparent much later. Our observations suggest that the M CFA oxidation pathway regulates the level of a signaling molecule that modulates the number of peroxisomal structures in a cell.

Key words: peroxisome • β-oxidation • peroxin • organelle multiplication • morphology

Introduction

Peroxisomes are subcellular organelles involved in a wide variety of metabolic processes. Their importance is underlined by the recognition of an increasing number of inherited disorders in which one or more peroxisomal functions are impaired (Wanders et al., 1995; Powers and Moser, 1998). The most severe peroxisomal disorders include the disorders of peroxisome biogenesis caused by a mutation in one of a variety of genes (P E X) encoding peroxisome biogenesis factors (peroxins; for nomenclature see Distel et al., 1996). The human PEX genes have their orthologues in simple model organisms such as Saccharomyces cerevisiae, which implies evolutionary conservation of the mechanism by which peroxisomes are formed and maintained in cells. Peroxisome number and volume can be regulated by environmental and intracellular factors. In vertebrates, peroxisomal proliferation has been shown to be regulated by peroxisomal proliferation activator receptors (P P A R)1, which are ligand-activated transcription factors (Issmann and Green, 1990; Dreyer et al., 1992; Lee et al., 1995). In addition, proper regulation of the size and morphology of the peroxisomal compartment is dependent on a functional peroxisomal β-oxidation system as a defect in one of the first two β-oxidation enzymes, including acyl-CoA oxidase or either the D- or L-bifunctional enzyme, has been shown to result in a low number of peroxisomes in human fibroblasts and murine hepatocytes (Poll-Thé et al., 1988; Fan et al., 1996; Suzuki et al., 1997; Chang et al., 1999; Qi et al., 1999; van Grunsven et al., 1999).

Since S. cerevisiae contains only 1-2 small peroxisomes

1Abbreviations used in this paper: GFP-PTS1, green fluorescent protein containing a peroxisomal targeting signal type 1; LBD, ligand-binding domain; LCFA, long-chain fatty acids; M CFA, medium-chain fatty acid; PPAR, peroxisomal proliferation activator receptors.
per cell under most conditions of growth, proliferation of peroxisomes (to 10–20/cell) and induction of the fatty acid β-oxidation machinery is required in order to grow on a fatty acid as sole carbon source. The heterodimeric transcription factor Pip2p (Rottensteiner et al., 1996)/Oaf1p (Rottensteiner et al., 1997; Karpichev and Small, 1998) is required for fatty acid-induced peroxisome proliferation and regulates the expression of proteins required for fatty acid oxidation, i.e., the β-oxidation enzymes, proteins required for transport of metabolites across the peroxisomal membrane, and components of metabolite shuttles (K al et al., 1999). Interestingly, Δoaf1/Δpip2p cells also fail to induce expression of the abundant peroxisomal membrane protein Pex11p, thereby implying that Pex11p is coregulated with the β-oxidation machinery. Previously, Pex11p has been implicated in the regulation of the number of peroxisomes (Erdmann and Blobel, 1995; M arshall et al., 1995). Y. east mutants lacking the PEX11 gene are unable to increase the number of peroxisomes when grown on oleate-containing media and instead accumulate a few (4–5) giant peroxisomes. On the other hand, cells overexpressing Pex11p exhibit a large number of small peroxisomal structures (Erdmann and Blobel, 1995; M arshall et al., 1995; Sakai et al., 1995). Proteins with a low amino acid sequence similarity (20%) have been found in a wide variety of eukaryotes. Overexpression studies of these homologues in K. netoplastida, and mammals have also been shown to affect peroxisome abundance which suggests that all these proteins are orthologues (Lorenz et al., 1998; Passreiter et al., 1998; Schrader et al., 1998). Taken together, these results suggest that Pex11p is involved in a process leading to fission or vesiculation of preexisting peroxisomes. A n interesting observation in support for such a role of Pex11p was made by Passreiter et al. (1998). R at Pex11px was shown to bind coatamer in vitro by virtue of its cytoplasmically exposed carboxy-terminal dilysine motif. Recruitment of coatamer by Pex11p has been proposed to initiate vesiculation of peroxisomes and thereby influence peroxisome proliferation (Passreiter et al., 1998). However, this dilysine motif is not conserved in other Pex11p homologues thereby raising doubt about the universality of the proposed mechanism for Pex11p-mediated peroxisomal proliferation.

Here we report that S. cerevisiae Pex11p is primarily involved in the oxidation of fatty acids, a process that is restricted to peroxisomes. Fatty acid oxidation in S. cerevisiae is mediated via the peroxisomal matrix enzymes acyl-CoA oxidase (Fox1p), the bifunctional protein (Fox2p) and 3-ketoacyl-CoA thiolase (Fox3p; Kunau et al., 1995). The peroxisomal membrane has been shown to form a permeability barrier for substrates and metabolites, in vivo (van R oermund et al., 1995), which predicts the presence of specialized transport systems that facilitate transport of substrates and metabolites across the peroxisomal membrane. Substrates for β-oxidation have been shown to enter peroxisomes via two different pathways (see Fig. 1; H ettma et al., 1996). M. CFA s enter peroxisomes as free acids and are subsequently activated via the peroxisomal acyl-CoA synthetase, Faa2p, whereas long-chain fatty acids (LCFAs) are activated outside peroxisomes and are subsequently imported via the peroxisomal A B C transporter Pat1p/Pat2p (Shani et al., 1995; H ettma et al., 1996; V erleur et al., 1997).

Here we describe that a defect in β-oxidation of fatty acids results in accumulation of only a few giant peroxisomal structures per cell, a phenotype that implies that fatty acid β-oxidation is required for proper peroxisome proliferation in S. cerevisiae. This prompted us to study whether Pex11p is directly involved in fatty acid oxidation.

Materials and Methods

Yeast Strains and Culture Conditions

The wild-type strain used in this study was S. cerevisiae BJ1991 (MATa, leu2, trp1, ura3-251, prb1-1122, pep4-3, gal2; j ones, 1977). The Δfaa2 and Δpat1 mutants have been described before (H ettma et al., 1996). Y. east transformants were selected and grown on minimal medium containing 0.67% yeast nitrogen base without amino acids (YNB-WO; Difco), supplemented with 0.3% glucose and amino acids (20 μg/ml) as needed. Liquid rich media used to grow cells for DNA isolation, subcellular fractionation, β-oxidation assays, immunogold electron microscopy and enzyme assays were composed of 0.5% potassium phosphate buffer, pH 6.0, 0.3% yeast extract, 0.5% peptone, and either 3% glycerol or 0.12% oleate/0.2% Tween 40. Before shifting to these media, the cells were grown on minimal 0.3% glucose or 2% glucose medium for at least 24 h. M inimal oleate medium contains YNB-WO supplemented with all amino acids and 0.12% oleate/0.2% Tween 40.

Disruption of the FOX1 and PEX11 Genes

The Δfox1 and Δpex11 deletion mutants were generated by one-step PCR-mediated gene disruption using the kanMX4 (W ach et al., 1994) as selectable marker. The PCR-derived disruption constructs comprised of the kanMX4 gene flanked by short regions of homology (50 bp) corresponding to the FOX1 and PEX11 5' and 3' noncoding region. The resulting PCR fragments were introduced into S. cerevisiae BJ1991 cells. G418-resistant clones were selected by growth on YPD plates containing 200 mg/liter G418 (G. metcalf; W ach, 1996).

Medium chain fatty acids Activated long-chain fatty acids Cytosol

Pex11p Faa2p Pat1p/Pat2p Membrane

Acyl-CoA Fox1p β-oxidation Matrix

Fox2p

Fox3p Acetyl-CoA

Figure 1. Schematic representation of fatty acid β-oxidation in S. cerevisiae. Fatty acids can enter peroxisomes via two pathways: medium-chain fatty acids enter as free fatty acids and require Pex11p and the peroxisomal membrane-associated acyl-CoA synthetase, Faa2p, for their activation inside peroxisomes. Long-chain fatty acids are activated outside peroxisomes and are transported across the peroxisomal membrane via the heterodimeric peroxisomal ABC transporter comprised of Pat1p and Pat2p (Pxa2p and Pxa1p).
Subcellular Fractionation and Nycodenz Gradient Equilibrium Density Analysis

Subcellular fractionation was performed as described by (van der Leij et al., 1992). Organelle pellets (25,000 g) were layered on top of 15–35% Nycodenz solutions containing 5 mM MES, pH 6.0, 1 mM EDTA, 1 mM KCl, and 8.5% sucrose. The sealed tubes were centrifuged for 2.5 h in a vertical rotor (MSE 8 × 35 ml) at 19,000 rpm at 4°C. Gradients fractionated into 14 fractions were analyzed for enzyme activity of various marker enzymes as described below.

Electron Microscopy

Oleate-induced cells were fixed with 2% paraformaldehyde (wt/vol) and 0.5% glutaraldehyde (wt/vol). Ultra-thin sections were prepared as previously described (Gould et al., 1990).

Enzyme Assays

β-Oxidation assays in intact cells were performed as previously described (van Roermund et al., 1998). Cells were grown overnight in media containing oleate to induce fatty acid β-oxidation. The β-oxidation activity in wild-type cells in each experiment was taken as reference (100%) and is expressed as the sum of CO₂ and water-soluble β-oxidation products produced. The activities of oleate-, palmitate-, myristate-, laureate-, and octanoate-β-oxidation in oleate-grown wild-type cells were 12.1 ± 1.5; 1.0 ± 0.3; 1.9 ± 0.2; 2.7 ± 0.6; 8.1 ± 1.7 nmol/h/mg protein, respectively. The β-oxidation activity in lysates measured with octanoate as substrate was 6.4 ± 0.5 nmol/min/mg protein.

3-Hydroxyacyl-CoA dehydrogenase activity was measured on a Cobas-Fara centrifugal analyzer by monitoring the acetoacetyl-CoA–dependent rate of NADH consumption at 340 nm (Wanders et al., 1992). Fumarase activity was measured on a Cobas-Fara centrifugal analyzer monitoring the APADH production at 365 nm. The reaction was started with 10 mM fumarate in an incubation mixture of 100 mM Tris (pH 9.0), 0.1% Triton X-100, 4 U/ml malate dehydrogenase (Boehringer) and 1 mM APAD for 5 min at 37°C. A cyt-CoA synthetase activity was measured essentially as described (Koll et al., 1994). A cyt-CoA synthetase activity in wild-type cells measured with octanoate as substrate was taken as reference (100%) and corresponds to 120 pmol/min/mg protein in intact cells and 3,560 pmol/min/mg protein in lysates. Protein concentrations were determined by the bicinchonic acid method (Smith et al., 1985).

Figure 2. Medium-chain fatty acid β-oxidation plays an important role in the regulation of peroxisomal morphology and abundance in S. cerevisiae. (A–C) Immunogold electronmicrograph showing peroxisomes in wild-type cells (A), the β-oxidation mutant Δfox1 (B), and in Δpex11 (C). Oleate-induced cells (>10 h) were labeled using anti-thiolase antibodies and protA-coated 10-nm gold particles. Bar, 0.25 μm. (D) Fluorescent structures labeled with green fluorescent protein containing a peroxisomal type 1 targeting signal (GFP-PTS1) in various S. cerevisiae β-oxidation mutants. Cells were grown on oleate-containing medium for 4 h. The number and morphology of the peroxisomes were observed by fluorescence microscopy. At least 100 cells were observed (in random fields) in each sample. Each experiment was performed at least two times, and the mean are shown by error bars. (E) Expression of Pex11p in wild-type and mutant cells. The Western blot shows the expression of PEX11p and catalase in cells grown on oleate and represents lysates of wild-type cells and β-oxidation mutants.
Results

MCFA Oxidation Is Required for Peroxisome Proliferation

In S. cerevisiae, peroxisome number and volume are regulated in response to changes in the carbon source of the growth medium. Cells grown on glucose contain only 1–2 small peroxisomes whereas cells grown on media containing long-chain fatty acids (oleate) contain 10–20 peroxisomes.

In human cells, a deficiency of one of the peroxisomal β-oxidation enzymes such as acyl-CoA oxidase (Poll-Thé et al., 1988) leads to a defect in the proliferation of this organelle. We tested whether a yeast β-oxidation mutant deficient in acyl-CoA oxidase (Δfox1) would also be disturbed in peroxisomal proliferation (see Fig. 1 for a simplified view of the peroxisomal β-oxidation system). Therefore, both wild-type and Δfox1 cells were grown in oleate-containing medium and peroxisomes were visualized by immunogold electron microscopy using 10-nm gold particles coupled to antibody raised against Fox3p (thiolase). This analysis revealed aberrant peroxisomal structures in Δfox1 cells, which were larger and frequently surrounded by multiple membranes (see Fig. 2, A–C). To follow the peroxisomal proliferation process during the transition from glucose- to oleate-containing medium, we used the GFP-based proliferation assay developed by Marshall et al. (1996) which allows visualization of peroxisomal structures in living S. cerevisiae cells. For this purpose, we expressed green fluorescent protein containing a peroxisomal targeting signal type 1 (GFP-PTS1) in fatty acid oxidation mutants, in which either the gene encoding acyl-CoA oxidase (Δfox1), the peroxisomal ABC transporter Pat1p (Δpat1) or the peroxisomal fatty acyl-CoA synthetase (Δfaa2) was deleted. Previously, we have shown that Faa2p is specifically required for MCFA β-oxidation, whereas Pat1p is required for β-oxidation of long-chain fatty acids (Fig. 1). We found that the β-oxidation mutants Δfaa2 and Δfox1, but not Δpat1 cells, showed less fluorescent structures per cell (Fig. 2 D). However, 4 h after the shift to oleate medium these structures seem to be larger and more intensely fluorescent (not shown).

Oleate-induced peroxisome proliferation in S. cerevisiae requires both induction of genes via the Oaf1p/Pip2p pathway and proper expression of the peroxisomal membrane protein Pex11p. Western blot analyses of total lysates from the β-oxidation mutants revealed that Pex11p and a marker protein for the Oaf1p/Pip2p induction pathway, catalase A, were induced in response to oleate to the same level as in wild-type cells (Fig. 2 E). These results imply an important role for MCFA β-oxidation in the regulation of the morphology of the peroxisomal compartment.

Figure 3. Octanoate β-oxidation in oleate-induced wild-type and Δpex11 cells. (A) β-oxidation in intact cells. (B) β-oxidation in lysates. The β-oxidation activity in wild-type cells was taken as reference (100%) and is expressed as the sum of [1-14C]CO2 and water-soluble β-oxidation products produced.

Figure 4. β-Oxidation enzymes are localized to the giant peroxisomes of Δpex11 cells. (A) Subcellular fractionation experiment showing that the β-oxidation activity is present in the 17,000-g pellet (P) fraction of oleate-grown Δpex11 cells. A cell-free homogenate (H) was fractionated into a 17,000-g pellet (P) fraction and supernatant (S). (B) The 17,000-g pellet (P) was further fractionated by Nycodenz equilibrium density gradient centrifugation (fractions 1–14). The β-oxidation (black bars), fumarase (▲) or 3-hydroxy-CoA dehydrogenase (•) activity in each gradient fraction is expressed as a percentage of the sum activity measured throughout the gradient. (M) Mitochondria and (P) peroxisomes indicate fractions with the highest fumarase or 3-hydroxy-CoA dehydrogenase activity, respectively. Fraction 1 is at the bottom of the gradient.
Pex11p Is Required for MCFA Oxidation

Since MCFAs β-oxidation is required for peroxisome proliferation, the peroxisome fission defect observed in Δpex11 cells might be the consequence of a disturbance in β-oxidation.

To test this hypothesis we analyzed oxidation of the (1-14C)-labeled MCF As octanoate (Fig. 3 A). We found that oxidation of this MCF A is impaired in intact Δpex11 cells, but importantly, rates of fatty acid oxidation in lysates were unaffected (Fig. 3 B). These results show that the activity of the β-oxidation enzymes themselves was unaffected.

The deficiency in octanoate β-oxidation in intact but not in lysed cells may be explained by (a) mislocalization of one of the β-oxidation enzymes or (b) a physical separation of the β-oxidation enzymes and one of the substrates (fatty acids, ATP etc.) or cofactors. Earlier studies have shown that Δpex11 cells are not disturbed in import of peroxisomal proteins (Erdmann and Blobel, 1995; Marshall et al., 1995). The data in Fig. 4 A are in line with these results since virtually all β-oxidation activity in a homogenate of Δpex11 cells was present in the crude organellar pellet. Subsequent fractionation of the organellar pellet by density gradient centrifugation revealed that the β-oxidation activity cofractionated with the peroxisomal marker 3HA D (Fig. 4 B). These data show that all β-oxidation enzymes are present in peroxisomes of Δpex11 cells and strongly suggest that Pex11p is required for transport of β-oxidation metabolites/substrates across the peroxisomal membrane. Subsequently, we tested the β-oxidation capacity of Δpex11 cells using fatty acids of various chain length. As shown in Fig. 5 A, β-oxidation of MCF As was as deficient in Δpex11 cells as in Δfox1 cells (<1% of control), whereas only a partial deficiency was found with fatty acids of longer chain length. We conclude that Pex11p is specifically required for β-oxidation of MCF As.

Previously, we have shown that fatty acids can enter peroxisomes either as free fatty acids or as acyl-CoA esters. MCF As, that enter peroxisomes as free fatty acids, depend on the peroxisomal acyl-CoA synthetase Faa2p for their activation to acyl-CoA esters. Although a small fraction of LCFA s enter peroxisomes as free fatty acids, most of the LCFA s are activated outside peroxisomes and rely on the heterodimeric ABC transporter (Pat1p/Pat2p) for entry into peroxisomes (Hettema et al., 1996). Inside peroxisomes both medium-chain and long-chain acyl-CoA esters are β-oxidized via the same set of enzymes (Fig. 1). The MCF A-specific β-oxidation defect observed in Δpex11 cells suggests that Pex11p functions in the Faa2p-dependent pathway. We constructed double mutants lacking the PEX11 gene and either the FA2 A gene or the PAT1 gene and measured MCF A - and LCFA - β-oxidation activity in these double mutants to test this hypothesis (Fig. 5 B). Indeed, Δpex11/Δpat1 cells show a block in both MCF A and LCFA β-oxidation, whereas Δpex11/Δfaa2 cells are specifically disturbed in MCF A β-oxidation. These results show that Pex11p functions in the same fatty acid entry pathway as Faa2p and in parallel to Pat1p. We conclude that Pex11p is required for β-oxidation of fatty acids that enter peroxisomes as free fatty acids.

We investigated whether the impaired oxidation of MCF A in Δpex11 cells is caused by a loss of Faa2p activity.
tivity 4 h after the shift to oleate medium (Fig. 7, A and B). Indeed, mislocalization of Faa2p to the cytosol partially rescues the MCFA β-oxidation defect observed in Δpex11 cells. The cytosolically-produced MCFA-CoA esters can now enter peroxisomes via the pathway for activated fatty acids as indicated by the inability of cytosolic Faa2p to rescue the MCFA β-oxidation defect in Δpex11/Δpat1 cells.

We conclude that Pex11p provides Faa2p with substrate or cofactor, probably by facilitating substrate or cofactor transport across the peroxisomal membrane.

**Pex11p Is Primarily Involved in MCFA Oxidation, which Secondarily Affects Peroxisomal Proliferation**

We performed a kinetic experiment where we transferred cells from glucose- to fatty acid–containing medium and followed β-oxidation after the transfer. Fig. 8 A shows that Δpex11 cells were not able to oxidize octanoate already shortly after the transfer. In parallel, peroxisome proliferation was followed using GFP-PTS1. In Δpex11 cells peroxisome number and size were indistinguishable from wild-type cells up to 2 h after the transfer to oleate-containing medium (Fig. 8 C). After prolonged incubation on oleate medium (10 h) the typical morphology of giant peroxisomes became apparent. Initially, induction of oleate β-oxidation was slightly affected (Fig. 8 B), and only at later timepoints (24 h) oleate β-oxidation decreased (not shown). The defect in oleate oxidation of Δpex11 cells 24 h after the shift explains the retarded growth on oleate medium and probably reflects a failure to segregate giant peroxisomes to daughter cells (Erdmann and Blobel, 1995).

Since overexpression of Pex11p has been shown to increase peroxisome number, we tested the effect of Pex11p overexpression on MCFA β-oxidation. 3 h after the shift of glucose-grown cells to oleate medium, we measured a twofold increase in MCFA β-oxidation activity in cells transformed with a multicopy vector (2 μm) containing the PEX11 gene (Fig. 9). This observation supports our hy-
hypothesis that Pex11p is involved in MCFA β-oxidation, a process required for peroxisome proliferation.

Discussion

Peroxisomes constitute a dynamic compartment in eukaryotic cells. Its size is determined by both external and internal factors. The molecular nature of these factors remains to be established. In S. cerevisiae the fatty acid β-oxidation enzymes are exclusively confined to peroxisomes. When provided with fatty acids as sole carbon source in the growth medium, the peroxisomal compartment is required at its full capacity and is enlarged compared with cells grown on other carbon sources. A number of processes must be involved in enlarging the peroxisomal compartment and in maintaining the increased number of organelles. Newly synthesized matrix and membrane (-associated) proteins must be recruited by peroxisomes. Genetic screens have identified a number of proteins involved in these processes and peroxisomal targeting signals have been defined. Much less is known as to how the enlarging peroxisomal membranes obtain their lipids, which proteins participate in the formation of new peroxisomes or how peroxisomes are properly segregated to daughter cells.

Characterization of Pex11p in both yeast and in mammalian cells has led to the proposal that it is involved in fission of larger peroxisomes into smaller ones. Cells that lack Pex11p accumulate a few large peroxisomes in contrast to cells in which Pex11p is overproduced which contain many small peroxisomes. Furthermore, it was shown that rat Pex11p was capable of binding coatomer in vitro suggesting that vesiculation of peroxisomes made use of a more general combination of factors required for vesiculation of other organelles.

Here we have reevaluated this proposed role of Pex11p and suggest another function for this peroxin. Our interest was raised by observations suggesting that formation of large peroxisomes could be the result of abnormal fatty acid metabolism. For instance, deficiencies of acyl-CoA oxidase (Poll-Thé et al., 1988; Fan et al., 1996; Chang et al., 1999) or the multifunctional enzymes D- or L-bifunctional enzyme (Suzuki et al., 1997; Chang et al., 1999; Qi et al., 1999; van Grunsven et al., 1999) in mammalian cells results in a reduced number of peroxisomes with enlarged volume and in mouse hepatocytes overexpressing the multi-drug transporter MDR2 large numbers of peroxisomes are occasionally seen (Mauad et al., 1994). These observations suggest that peroxisome proliferation is under control of a signaling pathway influenced by certain metabolic ligands. We have explored these facts in a more systematic way in S. cerevisiae in combination with morphological analysis of peroxisomes using electron microscopy and fluorescence light microscopy. All our experiments point to the impor-
tance of M CFA oxidation for peroxisome proliferation to occur. On the basis of previous work Pex11p would be expected to act at a late execution point in this putative signaling cascade. Surprisingly, our experiments rather suggest that Pex11p acts at a much earlier stage. This is based on the fact that Δpex11 cells first show a deficiency in M CFA oxidation and much later develop abnormal peroxisomal structures when shifted from glucose to oleate-containing medium. These results provide strong evidence against the model that Pex11p is directly involved in a vesiculation process (at least in S. cerevisiae). We rather favor the idea that Pex11p is part of a chain of events leading to the production of a signaling molecule responsible for modulation of the peroxisome proliferation process.

Pex11p is involved either directly or indirectly in getting M CFAa across the membrane since a Δpex11 mutant shows latency characteristic of a membrane-mediated process: deficiency of M CFA oxidation when membranes are intact but full capacity for M CFA oxidation in a detergent lysate in which membranes are dissolved. Indeed, one of the substrates is not able to reach the fatty acid CoA synthetase Faa2p which is associated with the inside of the peroxisomal membrane, since no MCFA-CoA esters are formed in Δpex11 cells. These characteristics qualify Pex11p as a transporter either for MCFAs or for essential cofactors involved in β-oxidation such as ATP, CoA etc. Interestingly, Pex11p shows extensive amino acid sequence similarity to the ligand-binding domain (LBD) of the nuclear hormone receptors (Barnett et al., 2000). The greatest similarity is found with the LBD s of PPARα, which are able to bind fatty acids. In analogy, Pex11p might contain a binding site for fatty acids which suggests a role in fatty acid transport across the peroxisomal membrane.

Unfortunately, the properties of ScPex11p are not completely in line with being a straightforward metabolite transporter. The primary amino acid sequence does not reveal obvious membrane spanning regions that could form a pore-like structure. There is also some controversy about its peroxisomal sublocalization. Pex11p of S. cerevisiae has been reported to be an integral membrane protein by one group and was shown to be inside peroxisomes, tightly associated with the matrix side of the peroxisomal membrane by another group (Erdmann and Blobel, 1995; Marshall et al., 1995, 1996). In our hands, ScPex11p behaved as a peripheral membrane protein as it was extractable from membranes by carbonate treatment (data not shown). Mammalian Pex11p is considered to be an integral membrane protein with NH2 and COOH termini protruding into the cytoplasm (Passreiter et al., 1998). Some of these studies made use of epitope-tagged versions of Pex11p which might have influenced its position. Further work is required to settle this issue which is crucial for further delimitation of the role of Pex11p in fatty acid β-oxidation.

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