Pex7p translocates in and out of peroxisomes in *Saccharomyces cerevisiae*

Devi M. Nair, P. Edward Purdue, and Paul B. Lazarow

Mount Sinai School of Medicine, New York, NY 10029

THE JOURNAL OF CELL BIOLOGY

Pex7p is the soluble receptor responsible for importing into peroxisomes newly synthesized proteins bearing a type 2 peroxisomal targeting sequence. We observe that appending GFP to Pex7p's COOH terminus shifts Pex7p's intracellular distribution from predominantly cytosolic to predominantly peroxisomal in *Saccharomyces cerevisiae*. Cleavage of the link between Pex7p and GFP within peroxisomes liberates GFP, which remains inside the organelle, and Pex7p, which exits to the cytosol. The reexported Pex7p is functional, resulting in import of thiolase into peroxisomes and improved growth of the yeast on oleic acid. These results support the "extended shuttle" model of peroxisome import receptor function and open the way to future studies of receptor export.

Introduction

Peroxisomal matrix proteins are synthesized in the cytoplasm and imported posttranslationally across the peroxisomal membrane. This is achieved by means of a branched pathway with each branch responsible for proteins with one of several types of peroxisomal targeting sequences (PTSs). There are two well-characterized classes of PTSs, the PTS1 and PTS2, that are recognized by their cognate, soluble receptors Pex5p and Pex7p, respectively (Fujiki, 2000; Sacksteder and Gould, 2000; Subramani et al., 2000; Purdue and Lazarow, 2001a; Titorenko and Rachubinski, 2001; Eckert and Erdmann, 2003). Pex7p, which has been cloned from yeasts and mammals, is of special interest for two reasons. First, human *PEX7* has been shown to be the defective gene in Rhizomelic chondrodysplasia punctata (Braverman et al., 1997; Motley et al., 1997; Purdue et al., 1997) and can also cause Refsum disease (van den Brink et al., 2003), classifying these severe disorders of peroxisomal biogenesis as diseases of protein targeting and emphasizing the critical role of PTS2 targeting in peroxisomal biogenesis. Second, extensive localization data indicate that Pex7p is a soluble, not a membrane bound, receptor (Marzioch et al., 1994; Zhang and Lazarow, 1995; Elgersma et al., 1998). This has led to a model in which Pex7p acts as a mobile receptor picking up cargo in the cytosol and dropping it off at the peroxisome membrane ("simple shuttle"). Pex7p has been shown to interact with the docking proteins Pex13p and Pex14p (Stein et al., 2002) but the details of the delivery of cargo across the peroxisome membrane remain unclear (Lazarow, 2003).

Meanwhile, it was discovered that Pex5p follows an "extended shuttle" mode of transport (Dammai and Subramani, 2001) wherein a mobile receptor enters the peroxisomes during the course of its normal functions and reemerges to the cytosol to carry out further rounds of import. One aspect merits further study (Kunau, 2001): it was not entirely certain whether Pex5p goes all the way inside peroxisomes or just pokes itself partially through the membrane. Pex5p recycling raises the possibility that Pex7p might also use an "extended shuttle" mechanism.

Pex7p's intracellular location appears to be affected by how it is tagged. In *Saccharomyces cerevisiae* and human fibroblasts, NH_2-terminally Myc-tagged Pex7p was found predominantly in the cytosol (Marzioch et al., 1994; Braverman et al., 1997), whereas Pex7p tagged at its COOH terminus with three copies of the HA epitope was found mainly inside the peroxisomes in *S. cerevisiae* (Zhang and Lazarow, 1995). Pex7p with a COOH-terminal GFP tag was localized to the peroxisomes in mammalian cells with substantial cell to cell variation in the amount of Pex7p-GFP in the cytosol (Ghys et al., 2002). Success in raising antibodies against Pex7p allowed Elgersma et al. (1998) to show in *P. pastoris* a predominantly cytosolic localization for endogenous as well as overexpressed untagged Pex7p, with a little detected in the peroxisomes. Mukai et al. (2002) reported that both endogenous and overexpressed Pex7p were predominantly cytosolic in CHO cells as well as human fibroblasts.

In this paper, we compare the localization of native Pex7p with COOH-tagged Pex7p in the same cells. Further-

---

more, we take advantage of the accumulation of COOH-tagged Pex7p within peroxisomes to investigate the export step apart from import. This offers a critical advantage over the Pex5p study where the intraperoxisomal accumulation of receptor was not observable.

Strategy

A Pex7p-GFP fusion protein is used, which is found to accumulate inside peroxisomes in vivo in *S. cerevisiae.* Hypothesis: Pex7p normally recycles from cytosol into peroxisomes and back out to the cytosol as it functions; peroxisomal accumulation of Pex7p-GFP results from an inhibition of reexport due to the COOH-terminal GFP. In this case, intraperoxisomal cleavage of the fusion protein would result in free GFP inside peroxisomes and free Pex7p which could be reexported. We look for both the cleavage products.

S. cerevisiae peroxisomes, unlike mammalian peroxisomes, have no known processing protease. Therefore, a viral, sequence-specific protease, tobacco etch virus protease (TEVP) has been expressed and targeted to the peroxisomal compartment. We appended an SKL tripeptide to the COOH terminus of TEVP to create a TEVP-SKL fusion that would accumulate in peroxisomes. Faber et al. (2001) have shown that the protease activity of TEVP-SKL is limited to the peroxisomal compartment. The TEVP cleavage site (cs) sequence (ENLYFQS) was engineered between Pex7p and GFP to form a Pex7p-cs-GFP fusion protein. This substrate was expressed in the Δ*pex7* knockout strain to avoid confusion with endogenous Pex7p.

In practice, the Pex7p-cs-GFP fusion showed some activity in importing the PTS2 protein, thiolase, but was much less active than native Pex7p. If the Pex7p recycling hypothesis is correct, we predict that coexpression of Pex7p-cs-GFP and peroxisome-localized protease will lead to the intraperoxisomal formation of free Pex7p that will be reexported to the cytosol, and function to catalyze PTS2 import. If the hypothesis is wrong, both free GFP and free Pex7p would remain inside the peroxisomes, with no improvement in function. Because the fusion protein certainly accumulates within peroxisomes, this experiment is a critical test of reexport.

Results and discussion

Endogenous Pex7p is predominantly cytosolic in *S. cerevisiae*

Polyclonal antibodies raised against *S. cerevisiae* Pex7p specifically recognize the protein by immunoblotting in wild-type W303 cells. No band of corresponding molecular mass is detected in the Δ*pex7* strain (Fig. 1 A). These antibodies also recognize a protein of ∼60 kD; it probably is in mitochondria (cell fractionation not depicted) and is not depleted in Δ*pex7* cells.

The intracellular distribution of native Pex7p in wild-type cells was determined by cell fractionation (Fig. 1 B). A small amount of Pex7p was found in the organelle pellet and the rest in the supernatant. Controls showed that peroxisomal enzymes, thiolase, and acyl-CoA oxidase (AOx), were recovered in the pellet, indicating that the peroxisomes were intact. In the absence of Pex7p, as expected, the PTS2 protein thio-

lase was not packaged into peroxisomes, whereas AOx, which uses the PTS1 receptor Pex5p (Klein et al., 2002), was packaged normally (Fig. 1 B).

Pex7p-GFP is largely peroxisomal in *S. cerevisiae*

COOH-terminally tagged receptor, Pex7p-GFP, was expressed in *S. cerevisiae* and found by cell fractionation mostly in the organelle pellet. Although it is known that COOH-terminally tagged Pex7p accumulates in the peroxisomes, for an additional confirmation Pex7p-GFP was expressed in a strain lacking Pex14p, which is required for protein translocation into peroxisomes. In Δ*pex14* cells, Pex7p-GFP is almost exclusively in the supernatant (Fig. 1 C, top). Direct fluorescence microscopy for Pex7p-GFP showed a clear punctate fluorescence in the W303 strain, whereas Δ*pex14* cells expressing this
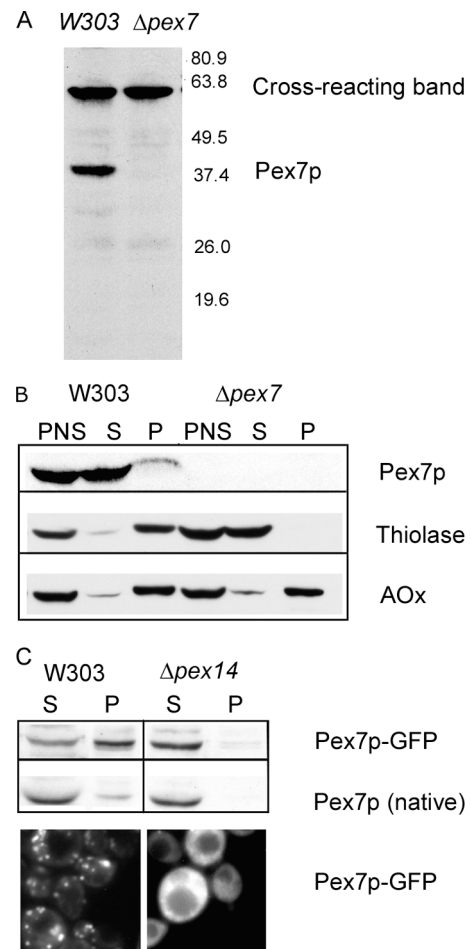

Figure 1. **Different subcellular distributions of native Pex7p and Pex7p-GFP.** (A) Rabbit antibodies against Pex7p recognize the endogenous protein in a total cell lysate of wild-type W303 cells by immunoblotting. (B) Subcellular distribution of endogenous Pex7p and peroxisomal enzymes in wild-type and Δ*pex7* cells. A postnuclear supernatant (PNS) fraction was centrifuged at 15,000 rpm for 20 min to separate an organelle pellet (P) from the cytosol-containing supernatant (S). Immunoblots of the peroxisomal enzymes thiolase and acyl-CoA oxidase (AOx) that use the Pex7p and Pex5p receptors, respectively, are included as controls. (C) Intracellular distribution of Pex7p-GFP fusion protein as well as native Pex7p in wild-type and Δ*pex14* cells. (Top) Cell fractionation and anti-Pex7p immunoblots. (Bottom) Fluorescence of the Pex7p-GFP.

protein show exclusive cytosolic fluorescence (Fig. 1 C, bottom). This confirms that the fluorescent particles in wild-type cells are indeed peroxisomes. It demonstrates that Pex14p, with which Pex7p is known to interact (Albertini et al., 1997), is essential for Pex7p entry into peroxisomes.

In this experiment the Pex7p-GFP fusion protein was expressed in wild-type cells with the endogenous Pex7p in order to compare directly their subcellular distributions. As shown in Fig. 1 C (middle) they are distinctly different: Pex7p was predominantly cytosolic just as it is in the absence of Pex7p-GFP, whereas Pex7p-GFP was largely peroxisomal. This clearly indicates that a COOH-terminal addition to Pex7p causes a remarkable change in the localization of the protein within the cell.

Pex7p-cs-GFP is also largely peroxisomal and is cleaved by TEVP

Pex7p-cs-GFP was partly cytosolic and partly peroxisomal when expressed in the Δ*pex7* strain, as expected (Fig. 2 A, right). The peroxisomal localization of Pex7p-cs-GFP was confirmed by colocalization of GFP fluorescence with thiolase immunofluorescence (not depicted). The fusion protein demonstrated limited function in catalyzing PTS2 protein import; a little thiolase was found in peroxisomes but most of it was cytosolic (Fig. 2 A, right).

As a control to prevent accumulation of Pex7p in the peroxisomes, a three–amino acid substitution (AIR → ELG at residues 285–287 of Pex7p) was used: it abolishes the targeting of Pex7p to peroxisomes and Pex7p's ability to catalyze PTS2 protein import and to support growth on oleic acid (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200407119/DC1).
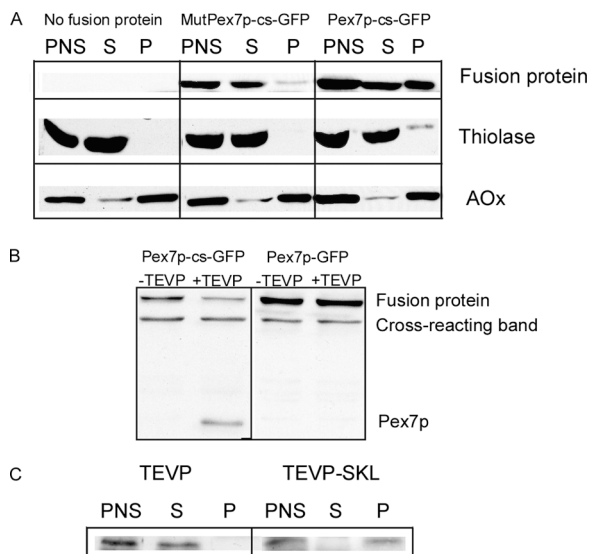

Figure 2. **Intracellular distribution and cleavability of Pex7p-cs-GFP.** (A) Cell fractionation of Δ*pex7* cells expressing Pex7p-cs-GFP (right) or MutPex7p-cs-GFP (middle) or no fusion protein (left). Immunoblots with anti-Pex7p, anti-thiolase, and anti-AOx antibodies. (B) In vitro cleavage of the fusion protein. Total cell lysates of Δ*pex7* cells, expressing either Pex7p-cs-GFP or Pex7p-GFP, were treated with or without purified TEVP (Invitrogen) for 30 min at 30°C. Pex7p cleavage product is seen only in the second lane that has Pex7p-cs-GFP treated with TEVP. (C) Intracellular distribution of TEVP and TEVP-SKL in Δ*pex7* cells. Immunoblot with anti-TEVP.

A mutant form of the fusion (MutPex7p-cs-GFP) with this substitution was found predominantly in the cytosol with very little detected in the organelle fraction (Fig. 2 A, middle). Consistent with this, no thiolase was packaged into peroxisomes. The normal AOx distribution confirms the peroxisome integrity in this experiment.

Pex7p-cs-GFP could be cleaved in vitro to form free Pex7p (Fig. 2 B) and free GFP (not depicted). Reactions lacking the protease established that no nonspecific cleavage occurred either in vivo or in vitro. Also, the Pex7p-GFP fusion protein that lacks the consensus cs was uncleaved, confirming the highly specific nature of TEVP action. Cell fractionation showed that TEVP-SKL was efficiently imported into peroxisomes with none detected in the cytosol and TEVP without the SKL tag remained cytosolic (Fig. 2 C), confirming the report of Faber et al. (2001).

Pex7p export from peroxisomes

No protease control. When Pex7p-cs-GFP is expressed alone in Δ*pex7* cells (without any viral protease), neither free Pex7p nor free GFP is detected (Fig. 3 A). The fusion protein shows a dual localization, part in peroxisomes and part in the cytosol, as observed previously. Only a little thiolase is found in the organelle pellet, which is consistent with the limited functionality of the fusion protein to catalyze PTS2 import into peroxisomes (Fig. 3 A).

Cytosolic cleavage. Coexpression of TEVP in the cytosol of these cells results in the cleavage of some of the cytosolic fusion protein, as shown by the appearance of free GFP exclusively in the supernatant fraction (Fig. 3 B). No GFP is detected in the organelle pellet. There is a concomitant appearance of free Pex7p in the supernatant, as expected. At long exposures, a little Pex7p (but no GFP) is apparent in the organelle pellet, suggesting that some of the Pex7p formed in the cytosol has entered the peroxisomes (unpublished data). There is also an increase in the amount of thiolase in the organelle pellet, indicating that the free Pex7p is functioning in PTS2 import.

Peroxisomal cleavage. TEVP-SKL in the peroxisomes of the Δ*pex7* cells also results in the cleavage of fusion protein, this time within the peroxisomes, as shown by the appearance of free GFP in the organelle pellet (Fig. 3 C). Again there is a concomitant appearance of free Pex7p. However, instead of being found in the organelle pellet where it was produced, together with the free GFP, the free Pex7p is observed in the supernatant fraction, indicating that it has been exported from the peroxisomes. At long exposures, a little Pex7p is also seen in the peroxisomes. The functioning of the free Pex7p generated within peroxisomes is shown by the fact that there is now more thiolase in the peroxisomes than in the supernatant (Fig. 3 C), whereas the reverse was true with TEVP (B).

Mutated fusion protein control. As a further check that TEVP-SKL activity is limited to the inside of the peroxisomes, MutPex7p-cs-GFP that remains in the cytosol was coexpressed with the peroxisome-targeted TEVP-SKL (Fig. 3 D). As expected, because the substrate and enzyme are in different cell compartments, only a trace of cleavage was observed: tiny amounts of free GFP and free Pex7p are seen in the super-

Figure 3. **Pex7p export from peroxisomes.** Δ*pex7* strains coexpressing Pex7p-cs-GFP without protease (A), with TEVP (B), or with TEVP-SKL (C) were analyzed by cell fractionation followed by SDS-PAGE and immunoblotting with anti-Pex7p (a), anti-GFP (b), anti-thiolase (c), and anti-AOx (d) antibodies. (D) MutPex7p-cs-GFP with TEVP-SKL.
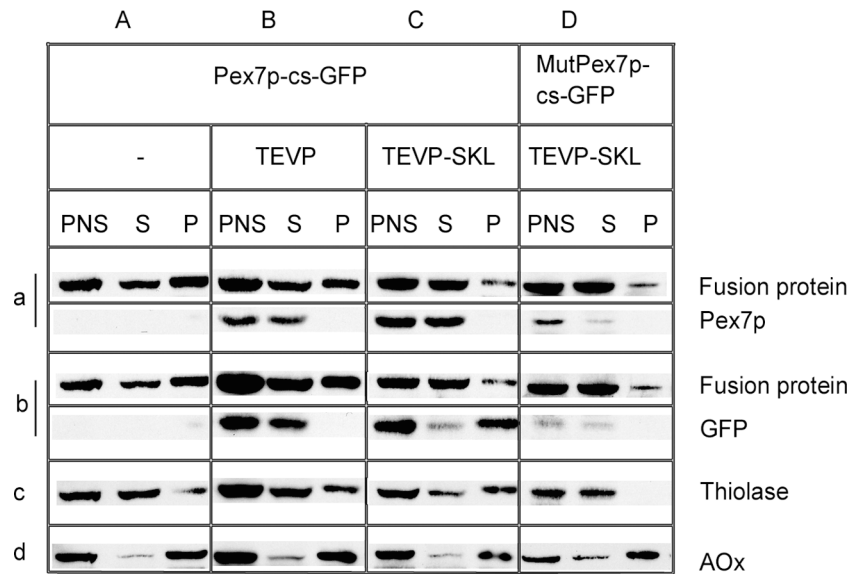
natant fraction. Perhaps this limited cleavage is due to the action of newly synthesized protease as it traverses the cytosol en route to the peroxisomes. In any case, this control confirms that most if not all of the cleavage seen in Fig. 3 C occurred in peroxisomes, and was not due to protease in transit through the cytosol.

In this experiment, AOx was consistently found in the organelle pellet, confirming that peroxisomal integrity was maintained during the cell fractionations.

Free Pex7p generated in vivo supports growth on oleic acid. A functional peroxisomal β-oxidation system is required for *S. cerevisiae* to grow on oleic acid (for review see Lazarow and Kunau, 1997). The Δ*pex7* strain lacks this ability because thiolase is not targeted to peroxisomes in the absence of the PTS2 receptor, Pex7p. Consistent with the Western blot data for thiolase (Fig. 3 A), the Δ*pex7* strain expressing the fusion protein alone showed detectable but slow growth compared with wild-type yeast (Fig. 4). The rate of growth increased with the coexpression of TEVP in the cytosol, and was larger still when TEVP-SKL was expressed in peroxisomes. These growth data correlate with an increase in free Pex7p produced and with the proportion of thiolase in peroxisomes (Fig. 3, compare B with C). They demonstrate that the Pex7p cleaved from the fusion protein is functional. The greater cleavage in the case of TEVP-SKL expression may be attributed to the concentration of substrate and protease together in the small peroxisome compartment.

Pex7p translocation follows an "extended shuttle" mechanism. These results strongly support the "extended shuttle" model of PTS receptor function. We demonstrate bimodal distributions of native Pex7p and Pex7p-GFP in *S. cerevisiae*. Both are present in peroxisomes and in the cytosol, but the proportions differ markedly: the native Pex7p is mostly in the cytosol, whereas the Pex7p-GFP is largely in the peroxisomes in the very same cells. This extends previous reports that native Pex7p is largely cytosolic in *P. pastoris* (Elgersma et al., 1998) and in CHO cells and human fibroblasts (Mukai et al., 2002). It is consistent with our previous observation that Pex7p with three HA epitopes on its COOH

terminus is mostly in peroxisomes (Zhang and Lazarow, 1995) and more recent observations that Pex7p-GFP is in peroxisomes in CHO cells (Ghys et al., 2002). We demonstrated by immunoelectron microscopy that Pex7p-HA$_3$ is found throughout the interior volume of the organelle, not on the inner or outer surface of the membrane (Zhang and Lazarow, 1995). Pex7p-GFP is likewise inside mammalian peroxisomes, not on the surface, by indirect immunofluorescence microscopy with low concentrations of detergent (Ghys et al., 2002). We conclude that the proportion of Pex7p within the peroxisomes is markedly increased by a COOH-terminal addition.

We observe cleavage of Pex7p-cs-GFP within peroxisomes by TEVP-SKL. As expected, the free GFP that is generated remains inside the organelle. Remarkably, most or all of the Pex7p that is generated is exported to the cytosol and functions in PTS2 protein import, resulting in the transport of thiolase into the peroxisomes. This makes the β-oxidation pathway functional and permits the growth of the yeast on oleic acid. This export of Pex7p generated within peroxisomes strongly supports the idea that Pex7p is normally recycling into and back out of the organelle, as has been suggested before (Mukai et al.,
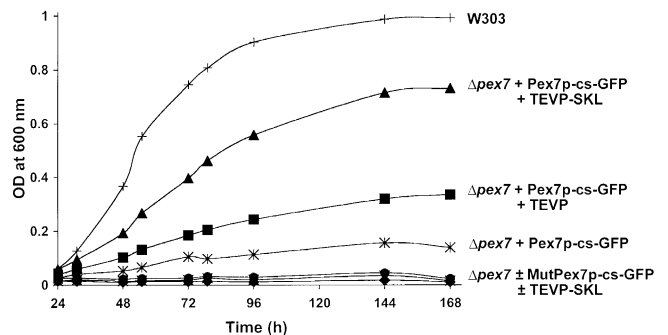

Figure 4. **Pex7p generated by fusion protein cleavage rescues growth of Δ*pex7* cells on oleic acid as sole carbon source.** Strains were precultured to mid-log phase in SCD and inoculated in 10 ml SCO liquid medium at a density of 10^5 cells/ml. Aliquots were withdrawn at the time points indicated and the OD read at 600 nm.

2002; Ghys et al., 2002). The accumulation of Pex7p-GFP within peroxisomes is thus not a complete artifact caused by the GFP, but rather a perturbation of the normal dynamic equilibrium between Pex7p entry and exit. Apparently, the presence of three HA moieties or GFP on the COOH terminus of Pex7p impairs its export from the peroxisome. This opens the way to future experiments on the mechanism of receptor reexport.

These results support and extend the conclusion of Subramani's group that Pex5p recycles into and out of peroxisomes in HeLa cells, which was the first clear evidence for the "extended shuttle" model (Dammai and Subramani, 2001). Those elegant experiments were gently criticized on the ground that they could not distinguish whether the Pex5p fusion protein just poked into the organelle far enough to be cleaved or fully entered the organelle (Kunau, 2001). Past and present experiments now demonstrate that Pex7p-HA$_3$, Pex7p-GFP, and GFP cleaved from Pex7p-cs-GFP accumulate inside peroxisomes and that Pex7p emerges. Together, these data indicate that Pex7p goes all the way inside and is reexported.

In the case of yeast, Pex7p traffics independently of Pex5p, in a complex with Pex18p and/or Pex21p (Purdue et al., 1998; Einwachter et al., 2001; Stein et al., 2002). In mammalian cells, Pex7p traffic into peroxisomes is dependent on the long form of Pex5p, with which it forms a complex, suggesting that the two receptors may enter and leave the organelle together (Otera et al., 2000; Dodt et al., 2001).

Materials and methods

Yeast strains, media, and growth conditions
Yeast strains (wild-type W303 and knockouts thereof) have been described previously (Purdue and Lazarow, 2001b). For induction of peroxisomes, cells were grown first to 5×10^7/ml in synthetic complete medium, SCD (0.67% yeast nitrogen base without amino acids plus 2% glucose, supplemented with an amino acid mixture lacking appropriate amino acids and/or uracil to maintain plasmids introduced into the yeast cells). The cells were inoculated at 5×10^6/ml in rich medium, YPEO (1% yeast extract, 2% peptone, 2% ethanol, 0.1% oleic acid, and 0.25% Tween 40) and grown for 18 h. To test growth with oleic acid as a sole carbon source, SCO medium was used (0.67% yeast nitrogen base without amino acids, 0.1% oleic acid, and 0.25% Tween 40 supplemented with amino acids, uracil, and adenine).

Cell fractionation and fluorescence microscopy
Spheroplasts were prepared, homogenized, and fractionated by differential centrifugation according to Thieringer et al. (1991). TEVP was inactivated during cell fractionation with 5 mM N-ethylmaleimide (Dougherty et al., 1989), which sufficed. Total cell lysates were made as described by Thieringer et al. (1991). Cell fractions and total lysates were analyzed by SDS-PAGE and immunoblotting (Zhang and Lazarow, 1996). Live cells in growth medium were analyzed for GFP fluorescence at RT with an Axiophot microscope (Carl Zeiss MicroImaging, Inc.) equipped with a plan-NEOFLUAR 100×, 1.3 NA, oil immersion objective lens (Carl Zeiss MicroImaging, Inc.). Images were recorded with a digital camera (Photometric Sensys) and IP lab software. They were assembled and labeled with Adobe photoshop.

Antibodies
Polyclonal rabbit antibodies against *S. cerevisiae* Pex7p (Purdue and Lazarow, 2001b) function in immunoblotting but are unable to detect either endogenous Pex7p or overexpressed Pex7p-GFP by immunofluorescence. Anti-thiolase, anti-AOx, and anti-TEVP antibodies were gifts from W.H. Kunau (Ruhr-Universität Bochum, Bochum, Germany), M. Skoneczny (Polish Academy of Sciences, Warsaw, Poland), and K. Nico Faber (University of Groningen, Haren, Netherlands), respectively. Anti-GFP (monoclonal) was purchased from Roche Molecular Biochemicals.

Miscellaneous
Standard techniques of yeast genetics (Sherman et al., 1986) and molecular biology (Ausubel et al., 1987) were used throughout.

Online supplemental material
The details of the construction of plasmids and the properties of the mutant Pex7p are given in the online supplemental material. Fig. S1 and online supplemental material are available at http://www.jcb.org/cgi/content/full/jcb.200407119/DC1.

References

Albertini, M., P. Rehling, R. Erdmann, W. Girzalsky, J.A.K.W. Kiel, M. Veenhuis, and W.H. Kunau. 1997. Pex14p, a peroxisomal membrane protein binding both receptors of the two PTS-dependent import pathways. *Cell.* 89:83–92.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1987. Current protocols in Molecular Biology. John Wiley & Sons, Inc., New York. 13.0.1–13.3.7.

Braverman, N., G. Steel, C. Obie, A. Moser, H. Moser, S.J. Gould, and D. Valle. 1997. Human *PEX7* encodes the peroxisomal PTS2 receptor and is responsible for rhizomelic chondrodysplasia punctata. *Nat. Genet.* 15:369–376.

Dammai, V., and S. Subramani. 2001. The human peroxisomal targeting signal receptor, Pex5p, is translocated into the peroxisomal matrix and recycled to the cytosol. *Cell.* 105:187–196.

Dodt, G., D. Warren, E. Becker, P. Rehling, and S.J. Gould. 2001. Domain mapping of human PEX5 reveals functional and structural similarities to *Saccharomyces cerevisiae* Pex18p and Pex21p. *J. Biol. Chem.* 276:41769–41781.

Dougherty, W.G., T.D. Parks, S.M. Cary, J.F. Bazan, and R.J. Fletterick. 1989. Characterization of the catalytic residues of the tobacco etch virus 49-kDa proteinase. *Virology.* 172:302–310.

Eckert, J.H., and R. Erdmann. 2003. Peroxisome biogenesis. *Rev. Physiol. Biochem. Pharmacol.* 147:75–121.

Einwachter, H., S. Sowinski, W.H. Kunau, and W. Schliebs. 2001. *Yarrowia lipolytica* Pex20p, *Saccharomyces cerevisiae* Pex18p/Pex21p and mammalian Pex5pL fulfil a common function in the early steps of the peroxisomal PTS2 import pathway. *EMBO Rep.* 2:1035–1039.

Elgersma, Y., M. Elgersma-Hooisma, T. Wenzel, J.M. McCaffery, M.G. Farquhar, and S. Subramani. 1998. A mobile PTS2 receptor for peroxisomal protein import in *Pichia pastoris. J. Cell Biol.* 140:807–820.

Faber, K.N., A.M. Kram, M. Ehrmann, and M. Veenhuis. 2001. A novel method to determine the topology of peroxisomal membrane proteins in vivo using the tobacco etch virus protease. *J. Biol. Chem.* 276:36501–36507.

Fujiki, Y. 2000. Peroxisome biogenesis and peroxisome biogenesis disorders. *FEBS Lett.* 476:42–46.

Ghys, K., M. Fransen, G.P. Mannaerts, and P.P. Van Veldhoven. 2002. Functional studies on human Pex7p: subcellular localization and interaction with proteins containing a peroxisome-targeting signal type 2 and other peroxins. *Biochem. J.* 365:41–50.

Klein, A.T., M. van den Berg, G. Bottger, H.F. Tabak, and B. Distel. 2002. *Saccharomyces cerevisiae* acyl-CoA oxidase follows a novel, non-PTS1, import pathway into peroxisomes that is dependent on Pex5p. *J. Biol. Chem.* 277:25011–25019.

Kunau, W.H. 2001. Peroxisomes: the extended shuttle to the peroxisome matrix. *Curr. Biol.* 11:R659–R662.

Lazarow, P.B. 2003. Peroxisome biogenesis: advances and conundrums. *Curr. Opin. Cell Biol.* 15:489–497.

Lazarow, P.B., and W.-H. Kunau. 1997. Peroxisomes. *In* Yeast III. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 547–605.

Marzioch, M., R. Erdmann, M. Veenhuis, and W.-H. Kunau. 1994. *PAS7* encodes a novel yeast member of the WD-40 protein family essential for import of 3-oxoacyl-CoA thiolase, a PTS2-containing protein, into peroxisomes. *EMBO J.* 13:4908–4917.

Motley, A.M., E.H. Hettema, E.M. Hogenhout, P. Brites, A.L.M.A. Ten Asbroek, F.A. Wijburg, F. Baas, H.S. Heijmans, H.F. Tabak, R.J.A. Wanders, and B. Distel. 1997. Rhizomelic chondrodysplasia punctata is a peroxisomal protein targeting disease caused by a non-functional PTS2 receptor. *Nat. Genet.* 15:377–380.

Mukai, S., K. Ghaedi, and Y. Fujiki. 2002. Intracellular localization, function,

and dysfunction of the peroxisome-targeting signal type 2 receptor, Pex7p, in mammalian cells. *J. Biol. Chem.* 277:9548–9561.

Otera, H., T. Harano, M. Honsho, K. Ghaedi, S. Mukai, A. Tanaka, A. Kawai, N. Shimizu, and Y. Fujiki. 2000. The mammalian peroxin Pex5pL, the longer isoform of the mobile peroxisome targeting signal (PTS) type 1 transporter, translocates the Pex7p•PTS2 protein complex into peroxisomes via its initial docking site, Pex14p. *J. Biol. Chem.* 275:21703–21714.

Purdue, P.E., and P.B. Lazarow. 2001a. Peroxisome biogenesis. *Annu. Rev. Cell Dev. Biol.* 17:701–752.

Purdue, P.E., and P.B. Lazarow. 2001b. Pex18p is constitutively degraded during peroxisome biogenesis. *J. Biol. Chem.* 276:47684–47689.

Purdue, P.E., J.W. Zhang, M. Skoneczny, and P.B. Lazarow. 1997. Rhizomelic chondrodysplasia punctata is caused by deficiency of human PEX7, a homologue of the yeast PTS2 receptor. *Nat. Genet.* 15:381–384.

Purdue, P.E., X. Yang, and P.B. Lazarow. 1998. Pex18p and Pex21p, a novel pair of related peroxins essential for peroxisomal targeting by the PTS2 pathway. *J. Cell Biol.* 143:1859–1869.

Sacksteder, K.A., and S.J. Gould. 2000. The genetics of peroxisome biogenesis. *Annu. Rev. Genet.* 34:623–652.

Sherman, F., G.R. Fink, and J.B. Hicks. 1986. Methods inYeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 186 pp.

Stein, K., A. Schell-Steven, R. Erdmann, and H. Rottensteiner. 2002. Interactions of Pex7p and Pex18p/Pex21p with the peroxisomal docking machinery: implications for the first steps in PTS2 protein import. *Mol. Cell. Biol.* 22:6056–6069.

Subramani, S., A. Koller, and W.B. Snyder. 2000. Import of peroxisomal matrix and membrane proteins. *Annu. Rev. Biochem.* 69:399–418.

Thieringer, R., H. Shio, Y. Han, G. Cohen, and P.B. Lazarow. 1991. Peroxisomes in *Saccharomyces cerevisiae*: Immunofluorescence analysis and import of catalase A into isolated peroxisomes. *Mol. Cell. Biol.* 11:510–522.

Titorenko, V.I., and R.A. Rachubinski. 2001. The life cycle of the peroxisome. *Nat. Rev. Mol. Cell Biol.* 2:357–368.

van den Brink, D.M., P. Brites, J. Haasjes, A.S. Wierzbicki, J. Mitchell, M. Lambert-Hamill, J. de Belleroche, G.A. Jansen, H.R. Waterham, and R.J. Wanders. 2003. Identification of PEX7 as the second gene involved in Refsum disease. *Am. J. Hum. Genet.* 72:471–477.

Zhang, J.W., and P.B. Lazarow. 1995. *PEB1* (*PAS7*) in *Saccharomyces cerevisiae* encodes a hydrophilic, intra-peroxisomal protein that is a member of the WD repeat family and is essential for the import of thiolase into peroxisomes. *J. Cell Biol.* 129:65–80.

Zhang, J.W., and P.B. Lazarow. 1996. Peb1p (Pas7p) is an intraperoxisomal receptor for the NH$_2$-terminal, type 2, peroxisomal targeting sequence of thiolase: Peb1p itself is targeted to peroxisomes by an NH$_2$-terminal peptide. *J. Cell Biol.* 132:325–334.