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The cytosolic and membrane components required for peroxisomal protein import

S. R. Terlecky, W. M. Nuttley and S. Subramani*

Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla (California 92093-0322, USA), Fax +1 619 534 0053, e-mail: sterlecky@ucsd.edu

Abstract. Peroxisomes are vital intracellular organelles which house enzymes involved in a variety of metabolic pathways. The large number of human disorders associated with flawed peroxisome biogenesis emphasizes the importance of protein targeting to, and translocation across, the peroxisomal membrane. This brief review will summarize some of the emerging themes of peroxisomal protein import, specifically addressing the targeting signals possessed by constituent proteins, as well as the cytosolic, membrane and luminal components of the import machinery. Although a detailed understanding of the molecular mechanisms of peroxisomal protein import is not yet available, remarkable progress has been made in the field in recent years. An overview of these advances will be presented.

Key words. Peroxisomes; biogenesis; targeting; receptors; import; heat shock proteins.

Peroxisomal targeting signals

Proteins destined for the peroxisome matrix contain specific molecular determinants which are recognized posttranslationally [1]. Most peroxisomal proteins contain the tripeptide serine-lysine-leucine, or a related variant at their carboxy-termini [1]. This peroxisomal targeting signal, called PTS1, targets proteins to the peroxisome in a variety of organisms across the evolutionary spectrum. Although context and species variations have been demonstrated, the general consensus motif appears to be a small residue (e.g. serine/alanine) at position -3, a basic residue (e.g. lysine/arginine) at position -2 and a hydrophobic residue (e.g. leucine/methionine) at the carboxy-terminus. PTS1 must be at the carboxy-terminus; it does not function at internal locations in the primary structure of a protein, or if the (negative) charge on the ultimate amino acid is altered [1]. Although a protein responsible for the direct recognition of PTS1 has been identified [2, 3], the mechanism by which this 'receptor' protein recognizes the PTS1 motif at the molecular level awaits elucidation.

Peroxisomal 3-ketoacyl-CoA thiolases from rat [4, 5], *Pichia pastoris* (A. Koller and S. Subramani, unpublished observations), and *Saccharomyces cerevisiae* [6, 7], *S. cerevisiae* Pas6p [8], *Hansenula polymorpha* HpPex8p (Perlp) [9, 10] and amine oxidase [11], watermelon glyoxysomal malate dehydrogenase [12] and *Trypanosoma brucei* aldolase [13] all contain a conserved dipeptide near their aminotermini, followed by a non-conserved pentapeptide, and finally another conserved dipeptide. This nonapeptide sequence, arginine/lysine-leucine/isoleucine-X_5-histidine/glutamine-leucine, is necessary and sufficient for peroxisomal targeting of proteins, and is known as PTS2. In contrast to PTS1, PTS2 functions at internal locations (K. Faber and S. Subramani, unpublished observations) and in some cases is actually cleaved from the rest of the protein by a peroxisomal protease following import into the organelle [1]. As is the case for the PTS1-sequence, a PTS2-specific binding protein has been identified [14–17].

A few specific proteins enter the peroxisome matrix devoid of PTS1 and PTS2 sequences. In some, novel PTSs have been described [16, 18–20]. Further characterization of these signals should reveal their generality as well as whether or not they are recognized by receptor proteins. Certain protein subunits devoid of PTSs have been shown to be imported into peroxisomes simply by association with other subunits containing a PTS [21, 22].

Signature sequences for the peroxisomal targeting of membrane proteins have not yet been extensively defined. The *P. pastoris* peroxisomal membrane protein, PpPex3p (Pas2p), contains a basic stretch within a sequence of 40 amino acids at its aminoterminus which acts as a peroxisomal membrane targeting signal (or mPTS) [23]. This sequence directs a reporter protein to the peroxisomal membrane. The basis of this membrane association, however, remains unclear. The *Candida boidinii* peroxisomal membrane protein, PMP47, also contains a stretch of hydrophilic amino acids that is necessary and sufficient for peroxisomal membrane targeting [24]. This sequence, found on the matrix-facing loop between putative membrane spans 4 and 5 (of 6) of the holoprotein, targets heterologous proteins to the organelle membrane [24]. The absence of a hydrophobic transmembrane domain in these two mPTSs suggests the intriguing possibility that peroxisomal membrane proteins are targeted to the organelle by a matrix targeting signal, and that perhaps the trans-

* Corresponding author.

membrane domains are responsible for anchoring the molecules as they traverse the membrane.

PTS receptors

The identification of PTS receptors was accomplished through the complementation of yeast mutants with PTS-specific protein-targeting defects. Such mutants have been identified from pools of mutants selected for the inability to grow on carbon sources, including methanol and/or oleic acid, that require peroxisomes for their metabolism (reviewed in ref. 1).

The first such mutant is the *pex5* (*pas8*) strain of *P. pastoris* [2, 3]. This strain fails to target PTS1-containing proteins correctly, whereas the PTS2-containing enzyme, thiolase, is localized to the defective peroxisomal remnants (peroxisomal ghosts) found in this mutant. Functional complementation led to the cloning of the *PpPEX5* (*PAS8*) gene, which encodes a protein, PpPex5p (Pas8p), with a predicted molecular mass of 65,000 Da.

The most striking feature of the PpPex5p (Pas8p) coding sequence is the presence of seven tetratricopeptide repeat (TPR) motifs which comprise the carboxy-terminal half of the protein. PpPex5p (Pas8p) binds specifically to the PTS1 sequence with high affinity ($K_d \approx 500$ nM) in vitro [3]. The TPR domain of PpPex5p (Pas8p) (and of ScPex5p [Pas10p] [25], see below) has been shown to be responsible for the binding of PTS1 [3].

PpPex5p (Pas8p) homologues have been identified in other yeast species including *S. cerevisiae* ScPex5p (Pas10p) [26], *H. polymorpha* HpPex5p (Per3p/Pah2p) [27, 28], and *Yarrowia lipolytica* YlPex5p (Pay32p) [29], as well as in human HsPex5p (PTS1R/PXR1) [30–32]. Just as in the *Pppex5* (*pas8*) mutant, the loss of a functional PTS1-receptor molecule in these organisms results in the inability to import PTS1-containing proteins properly. Despite a clear agreement on the role of the PTS1-receptor in the import of peroxisomal matrix proteins, there is no such consensus regarding the intracellular location of this molecule. PpPex5p (Pas8p) is reported to be associated with the cytosolic face of the peroxisomal membrane [3], ScPex5p (Pas10p) is cytosolic [20], HpPex5p (Per3p/Pah2p) is cytosolic and also intraperoxisomal [27, 28] and YlPex5p (Pay32p) is principally intraperoxisomal [29]. The human homologue, HsPex5p (PTS1R/PXR1), is thought to be both cytosolic and partially peroxisomal [30, 31] as well as completely peroxisomal [32]. The PTS1-receptor is not the only protein whose localization is equivocal (see below).

A PTS2 receptor has recently been identified in *S. cerevisiae* [14–17]. This 42,000-Da protein, called ScPex7p (Pas7p/Peb1p), is, by virtue of a 43-amino acid repeat, a member of the WD (rich in tryptophan and aspartate) repeat (β-transducin) protein family. Interestingly, in other systems, evidence exists for an interaction of WD and TPR domains, raising the possibility of an interaction between the PTS1 and PTS2 receptors. Indeed, such an interaction has been documented for the *S. cerevisiae* receptors [17]. ScPex7p (Pas7p/Peb1p) clearly does interact with PTS2 [16, 17]; whether or not additional factors are involved in this binding, however, is not clear. With respect to the intracellular localization of ScPex7p (Pas7p/Peb1p), the molecule has been reported to be cytosolic and partially peroxisomal [17] and also completely intraperoxisomal [15, 16].

The discrepancies in the PTS1- and PTS2-receptor localizations are puzzling. One interpretation is that the differences arise due to the varied conditions and methods employed to localize the molecules. However, a more unifying (and satisfactory) explanation is that the receptors shuttle from the cytosol to the peroxisome membrane, and perhaps on into the organelle lumen. This idea, that peroxisomal receptors bind their ligand in the cytosol and deliver them to the peroxisomal membrane translocation site, has already been suggested [33]. What is needed at this point is a definitive set of experiments designed to test whether or not the receptors (1) interact with components of the peroxisome membrane; (2) enter the peroxisome lumen; (3) are recycled out into the cytosol or are degraded inside the organelle.

With respect to point 1, a candidate docking protein for the PTS1 receptor has been described in *P. pastoris* and *S. cerevisiae* [34–36]. This 43,000-Da peroxisomal integral membrane protein, called Pex13p, interacts with the PTS1 receptor in both the two-hybrid and in vitro binding experiments. Interestingly, it is the SH3 domain of Pex13p that mediates this binding [34]. Whether or not this protein serves to dock the PTS2 receptor remains to be determined.

Finally, several peroxisomal proteins contain highly conserved zinc-finger binding domains of unknown function [37–40]. Recent biochemical experiments suggest that at least one zinc-binding protein appears to be directly involved in the docking of the PTS1 receptor to the peroxisome membrane (S. Terlecky/W. Nuttley and S. Subramani, unpublished observations). It remains to be seen whether or not this protein is identical to one of the previously described zinc-finger proteins [37–40]. The precise role zinc plays in the assembly of import complexes, as well as whether or not additional soluble or membrane components are involved, also awaits further characterization.

Translocation state

One of the most exciting recent developments in the peroxisome field has been the discovery that the import of peroxisomal proteins occurs without extensive un-

folding. Support for this hypothesis comes from several observations. First, there is the demonstration that proteins may be targeted to the peroxisome as oligomers. Specifically, in *S. cerevisiae*, a thiolase molecule lacking its PTS is imported into peroxisomes by virtue of its interaction as a dimer with an unmodified, i.e. native, thiolase subunit [21]. The suggestion is that the protein subunits dimerize in the cytosol and are imported together. In both yeast and mammalian cells, non-PTS-containing chloramphenicol acetyl transferase (CAT) monomers are also imported into peroxisomes by trimerizing with PTS-containing CAT monomers [22]. Certainly, the import of multimeric complexes suggests the absence of a radical unfolding requirement associated with translocation into peroxisomes.

Additional evidence comes from the observation that PTS1-coated gold particles as large as 9 nm are imported into human fibroblast peroxisomes, as are PTS-containing chemically stabilized proteins or antibodies [41]. This lack of an unfolding requirement during translocation suggests peroxisomal protein import more closely resembles import into the nucleus than it does import into the endoplasmic reticulum or mitochondria (see other reviews in this issue). However, the question remains as to how such seemingly large complexes are able to enter the peroxisome, especially considering the apparent absence of a large pore in the peroxisome membrane as well as the lack of evidence for endocytotic or pinocytotic invagination of the peroxisomal membrane. Perhaps peroxisomes simply contain a yet-to-be-described large or flexible translocation channel.

Chaperones

Despite the absence of global protein unfolding, there is clear evidence of a role for at least two heat shock proteins (hsps) in peroxisomal matrix protein import. Using a permeabilized-cell peroxisomal import system, both hsc73 [42] and hsp40 (W. Nuttley/S. Terlecky and S. Subramani, unpublished observations) have been shown to be necessary components of PTS1 import. Since hsps are ATP(adenosine triphosphate)ases, it is reasonable to suppose that they account, at least in part, for the ATP-hydrolysis requirement of peroxisomal matrix protein import [43, 44]. What role these and perhaps other chaperone proteins play in peroxisomal protein import is a matter of debate. Since the unfolding of proteins is not a tenable function per se, other options must be considered. Perhaps these chaperones function in the assembly or disassembly of translocation complexes, involving such components as the PTS receptors, PTS-containing substrates and proteins of the peroxisome membrane. The finding that hsc73 is recruited to the peroxisome membrane during import [42] is consistent with this view. It is interesting to note that the import of peroxisomal membrane proteins does not require ATP hydrolysis [45, 46]. The proteins or other factors required for peroxisomal membrane protein import are not yet known.

A role for the endoplasmic reticulum in peroxisome biogenesis?

When the import of proteins into the peroxisome is considered, the assumption is made that the organelle already exists. But how do peroxisomes arise? At one time, it was supposed that peroxisomes derived from ribosome-free regions of the endoplasmic reticulum [47]. Although the prevailing view is that peroxisomes are derived from pre-existing peroxisomes [48], recent evidence suggests a possible involvement of the endoplasmic reticulum in peroxisome biogenesis. One of the first clues was the finding that a rat liver 50,000-Da protein, PMP50, synthesized on membrane-bound polysomes, is associated with both endoplasmic reticulum and peroxisome membranes [49]. In addition, increased expression of the *S. cerevisiae* protein, Pas21p, causes a dramatic accumulation of endoplasmic reticulum-like membranes [50] and fusion of the first 16 amino acids of HpPex3p (Per9p), and a reporter protein is found in the endoplasmic reticulum membrane [51]. Could a peroxisomal

Table 1. Protein components of peroxisomal matrix protein import.

Protein	Function	Reference
Pex5p	binds PTS1-sequence	2, 3, 25, 30–32
Pex7p	binds PTS2-sequence	16, 17
hsc73	required for import (translocation complex assembly/disassembly?)	42
hsp40	required for import (translocation complex assembly/disassembly?)	W. Nuttley/S. Terlecky and S. Subramani, unpublished observations
Pex13p	Pex5p docking protein	34–36
Rat liver peroxisomal zinc-binding protein	Pex5p docking protein	S. Terlecky/W. Nuttley and S. Subramani, unpublished observations

membrane protein first insert into the endoplasmic reticulum membrane, and then perhaps bud off in vesicles that either fuse to form a peroxisome or fuse with pre-existing peroxisomes, allowing them to grow? Indeed, recent evidence from this laboratory supports a role for vesicles and vesicle fusion events in peroxisome biogenesis (J. Heyman/K. Faber and S. Subramani, unpublished observations).

Summary

In the near future, the precise functions of the proteins listed in table 1 will have to be elucidated, and components of the protein translocation machinery will have to be defined. It will be interesting to compare the requirements for import via the PTS1, PTS2 and mPTS pathways. An understanding of these import mechanisms will undoubtedly reveal important information regarding the evolution of this fascinating organelle.

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1 Subramani S. (1993) Protein import into peroxisomes and biogenesis of the organelle. Annu. Rev. Cell Biol. **9:** 445–478
2 McCollum D., Monosov E. and Subramani S. (1993) The *pas8* mutant of *Pichia pastoris* exhibits the peroxisomal protein import deficiencies of Zellweger syndrome cells: the PAS8 protein binds to the COOH-terminal tripeptide peroxisomal targeting signal, and is a member of the TPR protein family. J. Cell. Biol. **121:** 761–774
3 Terlecky S. R., Nuttley W. M., McCollum D., Sock E. and Subramani S. (1995) The *Pichia pastoris* peroxisomal protein PAS8p is the receptor for the C-terminal tripeptide peroxisomal targeting signal. EMBO J. **14:** 3627–3634
4 Swinkels B. W., Gould S. J., Bodnar A. G., Rachubinski R. A. and Subramani S. (1991) A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. EMBO J. **10:** 3255–3262
5 Osumi T., Tsukamoto T., Hata S., Yokota S., Miura S., Fujiki Y. et al. (1991) Amino-terminal presequence of the precursor of peroxisomal 3-ketoacyl-CoA thiolase is a cleavable signal peptide for peroxisomal targeting. Biochem. Biophys. Res. Commun. **181:** 947–954
6 Glover J. R., Andrews D. W., Subramani S. and Rachubinski R. A. (1994) Mutagenesis of the amino targeting signal of *Saccharomyces cerevisiae* 3-ketoacyl-CoA thiolase reveals conserved amino acids required for import into peroxisomes in vivo. J. Biol. Chem. **269:** 7558–7563
7 Erdmann R. (1994) The peroxisomal targeting signal of 3-oxoacyl-CoA thiolase from *Saccharomyces cerevisiae*. Yeast **10:** 935–944
8 Rehling P., Skaletz-Rorowski A., Franse M., Veenhuis M and Kunau W.-H. (1995) Pas6p or how to get into the peroxisome. Eur. J. Cell Biol. ECBO Meeting Heidelberg (Abstract)
9 Distel B., Erdmann R., Gould S. J., Blobel G., Crane D. I., Cregg J. M. et al. (1996) A unified nomenclature for peroxisome biogenesis. J. Cell Biol. **135:** 1–3
10 Waterham H. R., Titorenko V. I., Haima P., Cregg J. M., Harder W. and Veenhuis M. (1994) The *Hansenula polymorpha PER1* gene is essential for peroxisome biogenesis and encodes a peroxisomal matrix protein with both carboxy- and amino-terminal 14 targeting signals. J. Cell Biol. **127:** 737–749
11 Faber K. N., Keizer G. I., Pluim D., Harder W., Ab G. and Veenhuis M. (1995) The N-terminus of amine oxidase of *Hansenula polymorpha* contains a peroxisomal targeting signal. FEBS Lett. **357:** 115–120
12 Gietl C., Faber K. N., van der Klei I. J. and Veenhuis M. (1994) Mutational analysis of the N-terminal topogenic signal of watermelon glyoxysomal malate dehydrogenase using the heterologous host *Hansenula polymorpha*. Proc. Natl. Acad. Sci. USA **91:** 3151–3155
13 Blattner J., Dorsam H. and Clatyon C. E. (1995) Function of N-terminal import signals in trypanosome microbodies. FEBS Lett. **360:** 310–314
14 Marzioch M., Erdmann R., Veenhuis M. and Kunau W. H. (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:** 4908–4918
15 Zhang J. W. and Lazarow P. B. (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
16 Zhang J. W. and Lazarow P. B. (1996) Peb1p (Pas7p) is an 15 intraperoxisomal receptor for the NH_2-terminal, type 2, peroxisomal targeting signal of thiolase: Peb1p itself is targeted to peroxisomes by an NH_2-terminal peptide. J. Cell Biol. **132:** 325–334
17 Rehling P., Marzioch M., Niesen F., Wittke E., Veenhuis M. and Kunau W. (1996) The import receptor for the peroxisomal targeting signal 2 (PTS2) in *Saccharomyces cerevisiae* is encoded by the PAS7 gene. EMBO J. **15:** 2901–2913
18 Small G. M., Szabo L. J. and Lazarow P. B. (1988) Acyl-CoA oxidase contains two targeting sequences each of which can mediate protein import into peroxisomes. EMBO J. **7:** 1167–1173
19 Kragler F., Langeder A., Raupachova J., Binder M. and Hartig A. (1993) Two independent peroxisomal targeting signals in catalase A of *Saccharomyces cerevisiae*. J. Cell Biol. **120:** 665–673
20 Elgersma Y., van Roermund C. W., Wanders R. J. and Tabak H. F. (1995) Peroxisomal and mitochondrial carnitine acetyltransferases of *Saccharomyces cerevisiae* are encoded by a single gene. EMBO J. **14:** 3472–3479
21 Glover J. R., Andrews D. W. and Rachubinski R. A. (1994) 16 *Saccharomyces cerevisiae* peroxisomal thiolase is imported as a dimer. Proc. Natl. Acad. Sci. USA **91:** 10541–10545
22 McNew J. A. and Goodman J. M. (1994) An oligomeric protein is imported into peroxisomes in vivo. J. Cell Biol. **127:** 1245–1257
23 Wiemer E. A. C., Luers G., Faber K. N., Wenzel T., Veenhuis M. and Subramani S. (1996) Isolation and characterization of Pas2p, a peroxisomal membrane protein essential for peroxisome biogenesis in the methylotrophic yeast *Pichia pastoris*. J. Biol. Chem. **271:** 18973–18980
24 McNew J. A. and Goodman J. M. (1996) The targeting and assembly of peroxisomal proteins: some old rules do not apply. TIBS **21:** 54–58
25 Brocard C., Kragler F., Simon M. M., Schuster T. and Hartig A. (1994) The tetratricopeptide repeat-domain of the PAS10 protein of *Saccharomyces cerevisiae* is essential for binding the peroxisomal targeting signal-SKL. Biochem. Biophys. Res. Commun. **204:** 1016–1022

26 Van der Leij I., Franse M. M., Elgersma Y., Distel B. and Tabak H. F. (1993) PAS10 is a tetratricopeptide-repeat protein that is essential for the import of most matrix proteins into peroxisomes of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90:** 11782–11786
27 Van der Klei I. J., Hilbrands R. E., Swaving G. J., Waterham H. R., Vrieling E. G., Titorenko V. I. et al. (1995) The *Hansenula polymorpha PER3* gene is essential for the import of PTS1 proteins into the peroxisomal matrix. J. Biol. Chem. **270:** 17229–17236
28 Nuttley W. M., Szilard R. K., Smith J. J., Veenhuis M. and Rachubinski R. A. (1995) The *PAH2* gene is required for peroxisome assembly in the methylotrophic yeast *Hansenula polymorpha* and encodes a member of the tetratricopeptide repeat family of proteins. Gene **160:** 33–39
29 Szilard R. K., Titorenko V. I., Veenhuis M. and Rachubinski R. A. (1995) Pay32p of the yeast *Yarrowia lipolytica* is an intraperoxisomal component of the matrix protein translocation machinery. J. Cell Biol. **131:** 1453–1469
30 Dodt G., Braverman N., Wong C., Moser A., Moser H. W., Watkins P. et al. (1995) Mutations in the PTS1 receptor gene, PXR1, define complementation group 2 of the peroxisome biogenesis disorders. Nature Genet. **9:** 115–125
31 Wiemer E. A., Nuttley W. M., Bertolaet B. L., Li X., Francke U., Wheelock M. J. et al. (1995) Human peroxisomal targeting signal-1 receptor restores peroxisomal protein import in cells from patients with fatal peroxisomal disorders. J. Cell Biol. **130:** 51–65
32 Fransen M., Brees C., Baumgart E., Vanhooren J. C., Baes M., Mannaerts G. P. et al. (1995) Identification and characterization of the putative human peroxisomal C-terminal targeting signal import receptor. J. Biol. Chem. **270:** 7731–7736
33 Rachubinski R. A. and Subramani S. (1995) How proteins penetrate peroxisomes. Cell **83:** 525–528
34 Elgersma Y., Kwast L., Klein A., Voorn-Brouwer T., Van den Berg M., Metzig B., America T. et al. (1996) The SH3 domain of the *Saccharomyces cerevisiae* peroxisomal membrane protein Pex13p functions as a docking site for Pex5p, a mobile receptor for the import of PTS1 containing proteins. J. Cell Biol. **135:** 97–109
35 Erdmann R. and Blobel G. (1996) Identification of Pex13p, a peroxisomal membrane receptor for the PTS1 recognition factor. J. Cell Biol. **135:** 111–121
36 Gould S. J., Kalish J. E., Morell J. C., Bjorkman J., Urquhart A. J. and Crane D. I. (1996) An SH3 protein in the peroxisomal membrane is a docking factor for the PTS1 receptor. J. Cell Biol. **135:** 85–95
37 Tsukamoto T., Miura S. and Fujiki Y. (1991) Restoration by a 35K membrane protein of peroxisome assembly in a peroxisome-deficient mammalian cell mutant. Nature **350:** 77–81
38 Kalish J. E., Theda C., Morrell J. C., Berg J. M. and Gould S. J. (1995) Formation of the peroxisome lumen is abolished by loss of *Pichia pastoris* Pas7p, a zinc-binding integral membrane protein of the peroxisome. Mol. Cell Biol. **15:** 6406–6419
39 Waterham H. R., de Vries Y., Russel K. A., Xie W., Veenhuis M. and Cregg J. M. (1996) The *Pichia pastoris PER6* gene product is a peroxisomal integral membrane protein essential for peroxisome biogenesis and has sequence similarity to the Zellweger syndrome protein PAF-1. Mol. Cell Biol. **16:** 2527–2536
40 Kalish J. E., Keller G.-A., Morell J. C., Mihalik S. J., Smith B., Cregg J. M. et al. (1996) Characterization of a novel component of the peroxisomal protein import apparatus using fluorescent peroxisomal proteins. EMBO J. **15:** 3275–3285
41 Walton P. A., Hill P. E. and Subramani S. (1995) Import of stably folded proteins into peroxisomes. Mol. Biol. Cell **6:** 675–683
42 Walton P. A., Wendland M., Subramani S., Rachubinski R. A. and Welch W. J. (1994) Involvement of 70-kD heat-shock 20 proteins in peroxisomal import. J. Cell Biol. **125:** 1037–1046
43 Imanaka T., Small G. M. and Lazarow P. B. (1987) Translocation of acyl-CoA oxidase into peroxisomes requires ATP hydrolysis but not a membrane potential. J. Cell Biol. **105:** 2915–2922
44 Wendland M. and Subramani S. (1993) Cytosol-dependent peroxisomal protein import in a permeabilized cell system. J. Cell Biol. **120:** 675–685
45 Diestelkotter P. and Just W. W. (1993) In vitro insertion of the 22-kD peroxisomal membrane protein into isolated rat liver peroxisomes. J. Cell Biol. **123:** 1717–1725
46 Imanaka T., Shiina Y., Takano T., Hashimoto T. and Osumi T. (1996) Insertion of the 70-kDa peroxisomal membrane protein into peroxisomal membranes in vivo and in vitro. J. Biol. Chem. **271:** 3706–3713
47 Novikoff A. B. and Shin W. (1964) The endoplasmic reticulum in the Golgi zone and its relations to microbodies, Golgi apparatus and autophagic vacuoles in rat liver cells. J. Microscopie **3:** 187–206
48 Lazarow P. B. and Fujiki Y. (1985) Biogenesis of peroxisomes. Annu. Rev. Cell Biol. **1:** 489–530
49 Bodnar A. G. and Rachubinski R. A. (1991) Characterization of the integral membrane polypeptides of rat liver peroxisomes isolated from untreated and clofibrate-treated rats. Biochem. Cell Biol. **69:** 499–508
50 Elgersma Y. (1995) Transport of proteins and metabolites across the peroxisomal membrane in *Saccharomyxes cerevisiae*. Ph.D. Thesis, University of Amsterdam
51 Baerends R. J. S., Rasmussen S. W., Hilbrands R. E., van der Heide M., Faber K. N., Reuvekamp P. T. W. et al. (1996) The *Hansenula polymorpha PER9* gene encodes a peroxisomal membrane protein essential for peroxisome assembly and integrity. J. Biol. Chem. **271:** 8887–8894