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van der Klei, IJ; Veenhuis, M

Published in:
Current Opinion in Cell Biology

DOI:
10.1016/S0955-0674(02)00354-X

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2002

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
van der Klei, IJ., & Veenhuis, M. (2002). Peroxisomes: flexible and dynamic organelles. *Current Opinion in Cell Biology*, *14*(4), 500-505. https://doi.org/10.1016/S0955-0674(02)00354-X

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Peroxisomes: flexible and dynamic organelles

Ida J van der Klei* and Marten Veenhuis

Peroxisome development is a dynamic process that may involve organelle fusion and fission events. Cells contain different types of peroxisomes that vary in protein composition and capacity to incorporate membrane and matrix proteins. The protein import machinery is highly flexible and includes a cycling receptor that passes the peroxisomal membrane.

Addresses
Eukaryotic Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, PO Box 14, 9750 AA Haren, The Netherlands
*e-mail: I.J.van.der.Klei@biol.rug.nl

Current Opinion in Cell Biology 2002, **14**:500–505

0955-0674/02/$ – see front matter
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Abbreviations

COP	coat protein
mPTS	targeting signal of PMPs
PEX	gene encoding peroxin
Pexp	translation product of *PEX* gene
PMP	peroxisomal membrane protein
TPR	tetratricopeptide repeat

Introduction

Peroxisomes are ubiquitous cell organelles that strongly vary in abundance and function between cells. Characteristic is their extremely high matrix-protein concentration, sometimes leading to crystalline inclusions. The matrix is packed with enzyme molecules that catalyse diverse oxidative and biosynthetic reactions [1,2•]. Fungal Woronin bodies represent an exceptional class of peroxisomes in that they display a structural function in sealing septal pores of damaged hyphae [3] (Figure 1).

Because the peroxisomal membrane is semi-permeable *in vivo*, the metabolic function of the organelle requires the activities of several transporters. So far, however, only a few are known. Their identification is severely hampered by the exceptionally low abundance of peroxisomal membrane proteins (PMPs) [4]. The fact that peroxisomal membranes are leaky *in vitro* also obstructs their functional characterisation. Only recently, the first peroxisomal transporter was analysed for its transport activities and substrate specificities, which was achieved upon functional reconstitution in liposomes [5•].

The identification of proteins involved in peroxisome biogenesis has been more successful. During the past decade, various genes involved in matrix-protein import, membrane biogenesis, organelle fission and movement have been identified, and the first details on their molecular functions are emerging. Recent breakthroughs in this fascinating field are discussed in this paper.

Peroxisome development

The prevailing model of peroxisome biogenesis, proposed by Lazarow and Fujiki in 1985 [6], predicts that the organelle grows by uptake of new components from the cytosol and multiplies by division (Figure 2a). Little is known on the incorporation of lipids into the peroxisomal membrane. It has been postulated that they are transported from the ER to peroxisomes in vesicles, together with specific PMPs that reach peroxisomes via the ER (Figure 2b). Most PMPs, however, are thought to be sorted directly to the peroxisomal membrane [7••].

Different mPTSs — targeting signals of PMPs — have been identified [7••]. Interestingly, individual PMPs can contain more that one independent mPTS [8]. The mPTSs known so far lack conserved amino acid motifs but often contain a group of positively charged amino acids. Mutational analysis of the mPTS region of *Hansenula polymorpha* Pex3p revealed that only part of these charges are essential for targeting, however [9].

Three peroxins (Pex3p, Pex16p and Pex19p) have been implicated in PMP targeting/insertion [10–12]. Pex19p was initially proposed to be the mPTS receptor. Later studies challenged this view and pointed to a role for Pex19p in formation/maintenance of PMP complexes [13•,14•]. Moreover, the finding that in *Yarrowia lipolytica* peroxisomes are formed in the absence of Pex19p argues against a role for Pex19p as the mPTS receptor [15].

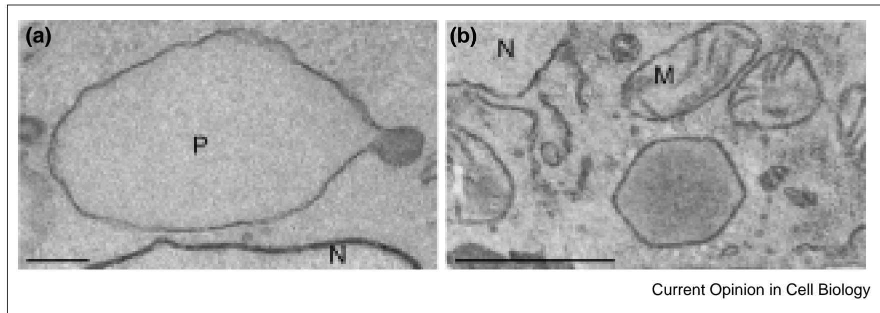
Matrix protein import

For matrix proteins, two peroxisomal targeting signals (PTS1 and PTS2) are known that are recognised by the cytosolic receptors Pex5p and Pex7p, respectively. Both receptors bind their cargo proteins in the cytosol and guide them to a docking site at the peroxisomal membrane. Most peroxisomal proteins contain a PTS1, and their import has been extensively studied, and it is most likely that the PTS2 pathway follows similar principles (for an excellent review, see [7••]).

Dammai and Subramani [16••] recently presented compelling evidence that human Pex5p is a cycling receptor, which, upon binding to a PTS1–cargo protein, associates with the peroxisomal membrane, translocates across it, and finally, upon release of its cargo, recycles to the cytosol (Figure 3). This so-called 'extended shuttle model' was first proposed for the yeast *H. polymorpha* [17,18]. Other evidence came from studies by Dodt and Gould [19], who showed that Pex5p molecules do cycle between the cytosol and peroxisomes *in vivo*.

The importance of the recent work of the Subramani laboratory [16••] is that it unequivocally showed that

Figure 1



Ultrathin sections of fungal cells showing **(a)** fission of a Woronin body from a normal peroxisome in *Penicillium chrysogenum* and **(b)** a hexagonal Woronin body in *Neurospora crassa.* $KMnO_4$ fixation. Bar = 0.5 µm. M, mitochondrion; N, nucleus; P, peroxisome.

Pex5p molecules functionally enter the organelle. This has major implications in that a protein-export machinery must exist for Pex5p, and that in case Pex5p dissociates from the inner surface of the membrane, a Pex5p sorting machinery exists in the organellar matrix. Both aspects are, as yet, completely unresolved. It is also not known whether import and export require separate machineries or use one and the same. In this context, it is tempting to speculate that some peroxins proposed to function in PTS1 import in fact may function in Pex5p export. Given the binding properties to Pex5p (see below), Pex13p is a plausible candidate. Another candidate is Pex8p, which might play a role in intraperoxisomal sorting of the PTS1 receptor to the export site [20].

Molecular mechanisms of Pex5p-dependent import

A remarkably high number of proteins specifically interact with Pex5p (Figure 3). PTS1 binds to tetratricopeptide repeats (TPRs) in the carboxy-terminal half of the protein. The three-dimensional structure of this domain of human Pex5p containing a PTS1 peptide revealed that two clusters of three TPRs (TPR1–3 and TPR 5–7) almost completely enclose the PTS1, while TPR4 forms a hinge region [21], which does not play a direct role in PTS1 binding [22].

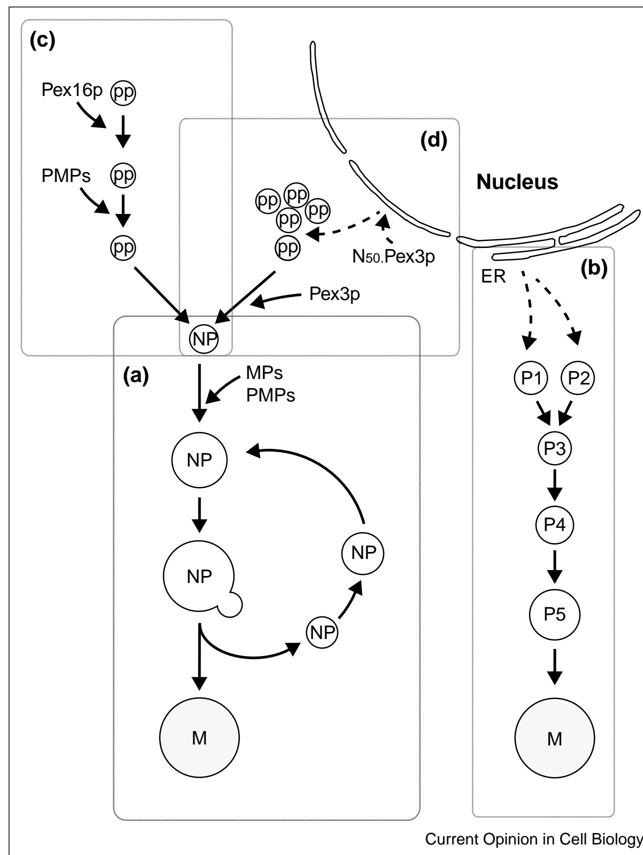
Pex8p, Pex12p, Pex13p and Pex14p interact with the amino-terminal half of Pex5p. Some of them also interact with each other or other peroxins, leading to an extensive network of interactions. There is still debate on the nature of these interactions. Some are only detected in one or a few species, and conflicting data have been presented on the domains involved. It seems unlikely that these discrepancies solely are related to species differences, as the mechanisms of peroxisome biogenesis are strongly conserved. Instead, they may reflect differences in experimental approaches. Indicative for this are the major differences observed in the strength of peroxin interactions. For instance, stable complexes containing Pex5p and several of its interacting partners could be isolated from rat and yeast peroxisomal membranes [23–25]. Other studies, however, pointed to dynamic, reversible interactions of Pex5p with other peroxins [26,27•].

According to the extended shuttle model, Pex5p undergoes a series of protein association and dissociation events during one cycle of PTS1 import. This implies that the affinity of Pex5p for the different peroxins must vary, and possibly depends on protein context, conformation or modifications (e.g. phosphorylation, as observed for Pex14p [28,29]). These changes in affinities may not (completely) occur under the experimental conditions used to analyse peroxin interactions (e.g. in two-hybrid studies, *in vitro* binding studies, or when small portions of the protein are used). Indeed, it has been reported that the interaction of Pex5p with only the Src homology 3 (SH3) domain of Pex13p is much stronger when only the SH3 domain is used relative to full-length Pex13p [26]. Also, the very strong interaction [30•] observed between small peptides of Pex5p and the amino-terminal domain of Pex14p is unlikely to occur *in vivo*, where Pex5p easily dissociates from the membrane upon altering the energy status of the cell [19].

Recent *in vitro* binding studies [26,27•] indicated that Pex5p interacts with various partner peroxins in a spatio-temporally differentiated manner: cargo-bound Pex5p had highest affinity for Pex14p, whereas unloaded Pex5p preferentially bound Pex13p. Other data suggest that binding of cargo-bound Pex5p causes dissociation of the Pex13p–Pex14p interaction [27•], which also points to a dynamic import machinery.

The order of the different Pex5p interactions is still highly speculative. Epistasis analysis revealed that Pex13p, Pex14p, Pex10p, Pex12p, Pex2p and Pex8p function at the initial stage of PTS1 import, followed by Pex1p/Pex6p and subsequently Pex22p and Pex4p [31••]. The latter is in line with previous data that revealed a role for Pex4p in Pex5p export and recycling [32]. Most current models group Pex13p, Pex14p and Pex17p as docking proteins and Pex2p, Pex10p and Pex12p as the actual protein translocation machinery (translocon). However, the experimental basis for these roles is still limited and mainly comes from the observation that human cells defective in Pex2p, Pex10p or Pex12p are able to recruit Pex5p to the peroxisomal membrane [33]. However, in *H. polymorpha* Pex14p is not essential for PTS1 import or Pex5p docking, but for the efficiency of

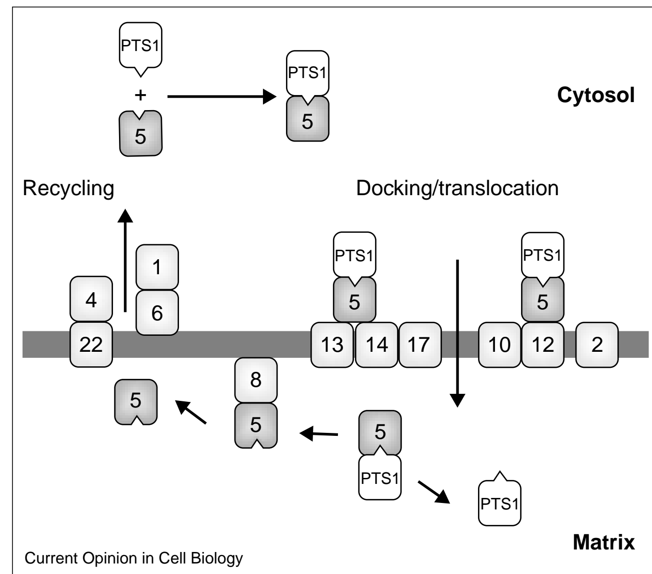
Figure 2



Hypothetical model containing different proposed pathways of peroxisome formation. **(a)** The classical growth and division model proposed by Lazarow and Fujiki [6], with the exception that the temporary matrix-protein import competence, as observed in yeast, is included [18]. This model predicts that newly synthesized peroxisomal matrix proteins (MPs) and membrane proteins (PMPs) are incorporated in pre-existing, nascent peroxisomes (NP). When a certain size is obtained, a new peroxisome buds off, resulting in the formation of a small nascent peroxisome together with a large, mature one (M) that has lost the capacity to take up additional matrix proteins. **(b)** The peroxisome maturation model proposed by Titorenko and Rachubinski [2•,44,45]. According to this model, preperoxisomal vesicles (P1 and P2) are derived from the ER, which fuse into P3 vesicles that develop via P4 and P5 into mature peroxisomes (M). Each type of organelle has specific properties with respect to protein import competence and protein composition [2•]. **(c)** The model of South and Gould [12] for re-introduction of peroxisomes into cells of Pex16p-deficient human cell lines. First, Pex16p is targeted to preperoxisomal structures (pp) that do not derive from the ER. Next, other PMPs are incorporated into these structures, which transforms the organelle into nascent matrix-protein import-competent peroxisomes. **(d)** A schematic representation of *H. polymorpha pex3* in which the first 50 amino-terminal residues of Pex3p (N_{50}.Pex3p) fused to green fluorescent protein (GFP) is produced, which leads to the formation of several small ‘preperoxisomal’ structures from the nuclear membrane [48•]. Upon subsequent production of full-length Pex3p, part of these structures is transformed into normal NPs.

PTS1 import [34]. Unexpectedly, *H. polymorpha* Pex14p has a dual role and functions both in peroxisome formation and in the opposite process: namely, selective peroxisome degradation [35•].

Figure 3



Schematic representation of the extended shuttle model of PTS1 protein import. Upon binding to a newly synthesised PTS1-containing cargo protein, Pex5p (5) associates with a putative docking site at the peroxisomal membrane, which may contain Pex13p (13), Pex14p (14) and Pex17p (17). Subsequently, this Pex5p–cargo complex is translocated across the membrane by a so far unknown mechanism that may involve the ring finger proteins Pex2p (2), Pex10p (10) and Pex12p (12). In the organellar matrix, the Pex5p–cargo complex dissociates, followed by export of Pex5p, processes that might be mediated by Pex8p (8), Pex1p/Pex6p (1/6) and Pex4p/Pex22p (4/22).

A plausible model for a dynamic PTS1 matrix-protein import machinery is that upon arrival of the Pex5p–cargo complex at the peroxisomal membrane, a docking complex assembles, which subsequently dissociates, paralleled by association of another subset of peroxins, into a dynamic translocation pore. At later stages, other complexes may be formed that are involved in cargo dissociation and Pex5p export/recycling. The complexes that are assembled and disassembled may contain common elements [36], which explains the extensive network of protein–protein interactions that have been discovered.

Differential PTS1 protein import?

Hypothetical models on PTS1 protein import generally propose a single import pathway. However, this route might be much more versatile. For instance, certain PTS1 proteins are only imported as oligomers, whereas translocation of others is restricted to the monomeric conformation [37•,38•]. This observation already divides the PTS1 pathway into two separate, but overlapping, tracks. It is most likely that there are additional overlapping PTS1 pathways. Illustrative for this are the differences observed in the import of the three major PTS1 proteins in mutant strains of methylotrophic yeasts (Table 1), where each protein apparently has its own requirements in addition to the general import components. Importantly, discrepancies on data of PTS1 protein import may therefore be related to differences in the marker

Table 1

Differential import of the three major PTS1 proteins in methylotrophic yeasts.

Protein	Mature conformation	Imported conformation	Wild type	Δ*pmp47*	*ass3*	Δ*pex14*	Δ*pex14::* P_{AOX}*PEX5*
AO	Octamer	Monomer	+	+	–	–	+
DHAS	Dimer	Dimer	+	-	+	–	+
CAT	Tetramer	?	+	+	+	–	–

In wild-type cells of methylotrophic yeasts (*Candida boidinii, H. polymorpha, Pichia pastoris*) the three major PTS1 proteins alcohol oxidase (AO), dihydroxyacetone synthase (DHAS) and catalase (CAT) are exclusively located in peroxisomes. AO is imported as a monomer, whereas DHAS is only imported as a dimer [38•]. *C. boidinii* cells lacking the transporter protein PMP47 (Δ*pmp47*) are specifically blocked in import of DHAS, whereas AO and CAT are normally imported [49]. In the *H. polymorpha pex14*-null mutant (Δ*pex14*), import of all three PTS1 proteins is blocked. However, upon overproduction of Pex5p (Δ*pex14::*P_{AOX}*PEX5*) import of AO and DHAS is restored, whereas CAT remains mislocated in the cytosol [34]. The recently isolated *H. polymorpha* AO assembly mutant (*ass3*) is specifically defective in AO import [50].

proteins used. In this respect, localisation experiments using artificial model proteins, such as green fluorescent protein (GFP)–SKL, should be interpreted with care.

Peroxisome division

Two proteins, Pex11p and VPS1, have been proposed to function in peroxisome fission. Pex11p overproduction leads to the formation of numerous small organelles, whereas in the absence of Pex11p only one large peroxisome is formed per cell [39,40]. It has been suggested that Pex11p functions in medium-chain fatty acid (MCFA) transport, rendering its role in proliferation as indirect [41]. This view is questioned, however, because the Pex11p-induced proliferation effect is also observed in cells that do not perform MCFA β-oxidation [42].

In *Saccharomyces cerevisiae*, the dynamin-related protein Vps1p was recently shown to be required for peroxisome division [43••]. It seems possible that Pex11p also participates in this process. Alternatively, Pex11p molecules might be involved in a coat-mediated budding process.

Alternative modes of peroxisome formation

In a great series of ground-breaking experiments, Titorenko and colleagues were the first to provide direct evidence that peroxisome development in *Y. lipolytica* involves membrane fusion [2•,44,45] (Figure 2b). These studies revealed that peroxisomes develop by a multistep process that starts with the formation of pre-peroxisomal vesicles, thought to arise from a subdomain of the ER. These structures harbour distinct subsets of membrane proteins, as well as components of coat protein II (COPII) vesicles, and transform into early peroxisomal precursors, designated P1 and P2, as a result of the uptake of additional membrane proteins and release of the COPII elements. P1 and P2 peroxisomes are competent to incorporate distinct sets of matrix proteins and fuse in a Pex1p/Pex6p-dependent way to generate P3 peroxisomes that develop into mature peroxisomes by a multistep assembly pathway via P4 and P5 peroxisomes (Figure 2b).

In peroxisome-deficient human fibroblasts also, evidence was obtained for a multistep peroxisome-assembly pathway to form new peroxisomes upon reintroduction of *PEX16* into cells of a Pex16p-defective cell line [12] (Figure 2c). In this system, first Pex16p is incorporated in a preperoxisome, followed by the insertion of other PMPs that enables subsequent matrix-protein import. These preperoxisomes are autonomous structures that do not arise from the ER and assemble into nascent peroxisomes independent of COP proteins [46] or the ER translocon [47].

Recently, however, it was shown that in *H. polymorpha pex3* cells the endomembrane system might serve as a template for the formation of new peroxisomes [48•]. Upon synthesis of the initial 50 amino acids of Pex3p (N_{50}.Pex3p) in *H. polymorpha pex3*, various vesicles where formed that arose from the nuclear envelope (Figure 2d). These vesicles showed peroxisomal characteristics and contained, apart from N_{50}.Pex3p, other peroxisomal membrane proteins. Upon subsequent synthesis of full-length Pex3p, a portion of these vesicles developed into normal peroxisomes [48•].

As concepts, the models proposed for *Y. lipolytica* (Figure 2b) and *H. polymorpha pex3* (Figure 2d) display comparable properties, as they suggest that (re-) introduction of peroxisomes initiates at the endomembrane system. However, the *Y. lipolytica* model (Figure 2b) proposes that this pathway occurs in wild-type cells upon induction of peroxisome formation. The re-introduction models (Figure 2c,d) explain how peroxisomes assemble in cells that were fully devoid of peroxisomes due to genetic defects. It is unclear whether such a mechanism is also operative in cells that grow normally at peroxisome-inducing conditions. In *H. polymorpha*, the 'normal' pathway of growth and division became operative upon prolonged cultivation in cells in which peroxisome formation initially was started by N_{50}.Pex3p-induced vesicles (Figure 2a,d). Thus, in this organism the above mechanism of peroxisome recovery may represent a rescue mechanism that becomes functional in case peroxisomes are lost — for example, owing to failure in inheritance.

Conclusions

Despite the progress made in the isolation and characterisation of various proteins essential for peroxisome biogenesis, our

knowledge on their precise function is still remarkably poor. New approaches, including biophysical techniques, are essential to resolve the molecular details of matrix-protein import and the origin and synthesis of the peroxisomal membrane. However, a major problem concerns the striking differences in the proposed functions for specific peroxins. As outlined by Purdue and Lazarow [7••] for the highly diverse functions suggested for Pex1p and Pex6p (vesicle fusion versus matrix-protein import), it is difficult to envisage that these discrepancies are simply due to species differences or are methodology related. A possible strategy to resolve these questions is to make room for a more prominent position of physiology in the field. At present, the bulk of the progress is based on molecular genetic approaches. However, especially for yeasts the physiology (growth conditions, substrates, growth phase) of the examined cells may differ largely. Obviously, these variations are reflected in cell performance and thus also in the kinetics of processes involved in peroxisome biogenesis. It seems therefore advisable for the field to normalise cultivation conditions, preferably by using chemostat cultures, as these predict cells of equal performance at various separate cultivations. The use of normalised cells also seems crucial for comparison of data of future genomic/proteomic approaches.

Acknowledgements

We apologise to those authors whose work was not cited for reasons of space limitations. We thank Klaas Sjollema for preparing Figure 1. IJVDK is supported by an NWO-PIONIER grant.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Van den Bosch H, Schutgens RB, Wanders RJ, Tager JM: **Biochemistry of peroxisomes.** *Annu Rev Biochem* 1992, **61**:157-197.

2. • Titorenko VI, Rachubinski RA: **The life cycle of the peroxisome.** *Nature Rev* 2001, **2**:357-368.
A comprehensive review detailing recent advances in peroxisome biogenesis, with emphasis on the multistep assembly pathway of peroxisomes in *Yarrowia lipolytica* and the role of the endomembrane in peroxisome formation.

3. Jedd G, Chua NH: **A new self assembled peroxisomal vesicle required for efficient resealing of the plasma membrane.** *Nat Cell Biol* 2000, **2**:226-231.

4. Schäfer H, Neu K, Sickmann A, Erdmann R, Meyer HE: **Identification of peroxisomal membrane proteins of *Saccharomyces cerevisiae* by mass spectrometry.** *Electrophoresis* 2001, **22**:2955-2968.

5. • Palmieri L, Rottensteiner H, Girzalsky W, Scarcia P, Palmieri F, Erdmann R: **Identification and functional reconstitution of the yeast peroxisomal adenine nucleotide transporter**. *EMBO J* 2001, **20**:5049-5059.
The first analysis of transport activities and substrate specificities of a peroxisomal transporter.

6. Lazarow PB, Fujiki Y: **Biogenesis of peroxisomes.** *Annu Rev Cell Biol* 1985, **1**:489-530.

7. •• Purdue PE, Lazarow PB: **Peroxisome biogenesis**. *Annu Rev Cell Dev Biol* 2001, **17**:701-752.
An excellent and extensive overview on our current knowledge of peroxisome biogenesis.

8. Jones JM, Morrell JC, Gould SJ: **Multiple distinct targeting signals in integral peroxisomal membrane proteins**. *J Cell Biol* 2001, **153**:1141-1150.

9. Baerends RJ, Faber KN, Kram AM, Kiel JA, van der Klei IJ, Veenhuis M: **A stretch of positively charged amino acids at the N terminus *of Hansenula polymorpha* Pex3p is involved in incorporation of the protein into the peroxisomal membrane.** *J Biol Chem* 2000, **275**:9986-9995.

10. Hettema EH, Girzalsky W, van den Berg M, Erdmann R, Distel B: ***Saccharomyces cerevisiae* Pex3p and Pex19p are required for proper localization and stability of peroxisomal membrane proteins.** *EMBO J* 2000, **19**:223-233.

11. Sacksteder KA, Jones JM, South ST, Li X, Liu Y, Gould SJ: **PEX19 binds multiple peroxisomal membrane proteins, is predominantly cytoplasmic, and is required for peroxisome membrane synthesis.** *J Cell Biol* 2000, **148**:931-944.

12. South ST, Gould SJ: **Peroxisome synthesis in the absence of pre-existing peroxisomes**. *J Cell Biol* 1999, **144**:255-266.

13. • Snyder WB, Koller A, Choy AJ, Subramani S: **The peroxin Pex19p interacts with multiple, integral membrane proteins at the peroxisomal membrane**. *J Cell Biol* 2000, **149**:1171-1178.
Evidence for a role of Pex19p as chaperone for peroxisomal membrane proteins (PMPs), based on the finding that Pex19p predominantly interacts at the peroxisomal membrane with cytosolic domains, but not the targeting signal of PMPs, of pre-existing PMPs.

14. • Fransen M, Wylin T, Brees C, Mannaerts GP, Van Veldhoven PP: **Human Pex19p binds peroxisomal integral membrane proteins at regions distinct from their sorting sequences**. *Mol Cell Biol* 2001, **21**:4413-4424.
In this paper, the Pex19p binding sites and the targeting signals of PMPs (peroxisomal membrane proteins) (mPTSs) of a large number of PMPs were defined. Thorough binding studies and mutational analysis revealed that the mPTSs and Pex19p binding domains of several of PMPs are distinct entities, indicating that Pex19p is not an mPTS receptor.

15. Lambkin GR, Rachubinski RA: ***Yarrowia lipolytica* cells mutant for the peroxisomal peroxin Pex19p contain structures resembling wild-type peroxisomes**. *Mol Biol Cell* 2001, **12**:3353-3364.

16. •• Dammai V, Subramani S: **The human peroxisomal targeting signal receptor, Pex5p, is translocated into the peroxisomal matrix and recycled to the cytosol.** *Cell* 2001, **105**:187-196.
Using well-designed, novel experimental approaches, compelling evidence is presented for the extended shuttle model of peroxisomal targeting signal 1 (PTS1) protein import. In this model, the PTS1 receptor, Pex5p, participates in multiple rounds of entry into peroxisomes and export to the cytosol.

17. Van der Klei IJ, Hilbrands RE, Swaving GJ, Waterham HR, Vrieling EG, Titorenko VI, Cregg JM, Harder W, Veenhuis M: **The *Hansenula polymorpha PER3* gene is essential for the import of PTS1 proteins into the peroxisomal matrix.** *J Biol Chem* 1995, **270**:17229-17236.

18. Van der Klei IJ, Veenhuis M: **A molecular analysis of peroxisome biogenesis and function in *Hansenula polymorpha*: a structural and functional analysis**. *Annals NY Acad Sci* 1996, **804**:47-59.

19. Dodt G, Gould SJ: **Multiple PEX genes are required for proper subcellular distribution and stability of Pex5p, the PTS1 receptor: evidence that PTS1 protein import is mediated by a cycling receptor.** *J Cell Biol* 1996, **135**:1763-1774.

20. Rehling P, Skaletz-Rorowski A, Girzalsky W, Voorn-Brouwer T, Fransen MM, Distel B, Veenhuis M, Kunau WH, Erdmann R: **Pex8p, an intraperoxisomal peroxin of *Saccharomyces cerevisiae* required for protein transport into peroxisomes binds the PTS1 receptor Pex5p**. *J Biol Chem* 2000, **275**:3593-3602.

21. Gatto GJ, Geisbrecht BV, Gould SJ, Berg JM: **Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5.** *Nat Struct Biol* 2000, **7**:1091-1095.

22. Klein AT, Barnett P, Bottger G, Konings D, Tabak HF, Distel B: **Recognition of peroxisomal targeting signal type 1 by the import receptor Pex5p.** *J Biol Chem* 2001, **276**:15034-15041.

23. Gouveia AMM, Reguena C, Oliveira MEM, Sa-Miranda C, Azevedo JE: **Characterization of peroxisomal Pex5p from rat liver.** *J Biol Chem* 2000, **275**:32444-32451.

24. Reguenga C, Oliveira ME, Gouveia AM, Sa-Miranda C, Azevedo JE: **Characterization of the mammalian peroxisomal import machinery: Pex2p, Pex5p, Pex12p, and Pex14p are subunits of the same protein assembly.** *J Biol Chem* 2001, **276**:29935-29942.

25. Albertini M, Girzalsky W, Veenhuis M, Kunau WH: **Pex12p of *Saccharomyces cerevisiae* is a component of a multi-protein**

complex essential for peroxisomal matrix protein import. *Eur J Cell Biol* 2001, **80**:257-270.

26. Urquhart AJ, Kennedy D, Gould SJ, Crane DI: **Interaction of Pex5p, the type 1 peroxisome targeting signal receptor, with the peroxisomal membrane proteins Pex14p and Pex13p.** *J Biol Chem* 2000, **275**:4127-4136.

27. • Otera H, Setoguchi K, Hamasaki M, Kumashiro T, Shimizu N, Fujiki Y: **Peroxisomal targeting signal receptor Pex5p interacts with cargoes and import machinery components in a spatiotemporally differentiated manner: conserved Pex5p WXXXF/Y motifs are critical for matrix protein import.** *Mol Cell Biol* 2002, **22**:1639-1655.

Extensive *in vitro* binding studies on the interactions between Pex5p, Pex7p, Pex13p and Pex14p.

28. Komori M, Kiel JAKW, Veenhuis M: **The peroxisomal membrane protein Pex14p of *Hansenula polymorpha* is phosphorylated *in vivo*.** *FEBS Lett* 1999, **457**:397-399.

29. Johnson MA, Snyder WB, Lin Cereghino J, Veenhuis M, Subramani S, Cregg JM: ***Pichia pastoris* Pex14p, a phosphorylated peroxisomal membrane protein, is part of a PTS-receptor docking complex and interacts with many peroxins**. *Yeast* 2001, **18**:621-641.

30. • Saidowsky J, Dodt G, Kirchberg K, Wegner A, Nastainczyk W, Kunau WH, Schliebs W: **The di-aromatic pentapeptide repeats of the human peroxisome import receptor PEX5 are separate high affinity binding sites for the peroxisomal membrane protein PEX14**. *J Biol Chem* 2001 **276**:34524-34529.

Detailed quantitative and mutational analysis of the interaction of human Pex5p and Pex14p using fluorescence spectroscopy and surface plasmon resonance spectroscopy, novel techniques in peroxisome research. The results indicate that Pex5p contains multiple di-aromatic pentapeptide motifs that each forms a high-affinity binding site for Pex14p.

31. •• Collins CS, Kalish JE, Morrell JC, McCaffery JM, Gould SJ. **The peroxisome biogenesis factors Pex4p, Pex22p, Pex1p, and Pex6p act in the terminal steps of peroxisomal matrix protein import.** *Mol Cell Biol* 2000, **20**:7516-7526.

Elegant epistasis approach based on the steady-state levels of Pex5p in different constructed *Pichia pastoris pex* strains, indicating that Pex4p, Pex22p, Pex1p and Pex6p function in late steps of the PTS1 protein import pathway.

32. Van der Klei IJ, Hilbrands RE, Kiel JAKW, Rasmussen SW, Cregg JM, Veenhuis M: **The ubiquitin-conjugating enzyme Pex4p of *Hansenula polymorpha* is required for efficient functioning of the PTS1 import machinery.** *EMBO J* 1998, **17**:3608-3618.

33. Chang CC, Warren DS, Sacksteder KA, Gould SJ: **PEX12 interacts with PEX5 and PEX10 and acts downstream of receptor docking in peroxisomal matrix protein import**. *J Cell Biol* 1999, **147**:761-774.

34. Salomons FA, Kiel JAKW, Faber KN, Veenhuis M, Van der Klei IJ: **Overproduction of Pex5p stimulates import of alcohol oxidase and dihydroxyacetone synthase in a *Hansenula polymorpha pex14* null mutant.** *J Biol Chem* 2000, **275**:12603-12611.

35. • Bellu AR, Komori M., Van der Klei IJ, Kiel JAKW, Veenhuis M: **Peroxisome biogenesis and selective degradation converge at Pex14p**. *J Biol Chem* 2001, **276**: 44570-44574.

First report on the essential role of a peroxin, Pex14p, in selective peroxisome degradation.

36. Snyder WB, Koller A, Choy AJ, Johnson MA, Cregg JM, Rangell L, Keller GA, Subramani S: **Pex17p is required for import of both peroxisome membrane and lumenal proteins and interacts with Pex19p and the peroxisome targeting signal-receptor docking complex in *Pichia pastoris***. *Mol Biol Cell* 1999, **10**:4005-4019.

37. • Titorenko VI, Nicaud JM, Wang H, Chan H, Rachubinski RA: **Acyl-CoA oxidase is imported as a heteropentameric, cofactor-containing complex into peroxisomes of *Yarrowia lipolytica***. *J Cell Biol* 2002, **156**:481-494.

Careful biochemical study demonstrating that acyl-CoA oxidase in *Yarrowia lipolytica* is only imported into peroxisomes when it is correctly assembled in the cytosol as a heteropentameric cofactor-containing complex. This work stresses the physiological significance of oligomeric protein import into peroxisomes.

38. • Stewart MQ, Esposito RD, Gowani J, Goodman JM: **Alcohol oxidase and dihydroxyacetone synthase, the abundant peroxisomal proteins of methylotrophic yeasts, assemble in different cellular compartments**. *J Cell Sci* 2001, **114**:2863-2868.

Another example (see Titorenko *et al.* [2002] [37•]) of a peroxisomal targeting signal 1 (PTS1) protein, dihydroxyacetone synthase, that is only imported as an oligomer. Interestingly, however, in the same cells another PTS1 protein, alcohol oxidase, is only imported as a monomer, indicating that PTS1 protein import is not confined to oligomers.

39. Erdmann R, Blobel G: **Giant peroxisomes in oleic acid-induced *Saccharomyces cerevisiae* lacking the peroxisomal membrane protein Pmp27p**.*J Cell Biol* 1995, **128**:509-523.

40. Marshall PA, Krimkevich YI, Lark RH, Dyer JM, Veenhuis M, Goodman JM: **Pmp27 promotes peroxisomal proliferation**. *J Cell Biol* 1995, **129**:345-355.

41. Van Roermund CW, Tabak HF, van Den Berg M, Wanders RJ, Hettema EH: **Pex11p plays a primary role in medium-chain fatty acid oxidation, a process that affects peroxisome number and size in *Saccharomyces cerevisiae***. *J Cell Biol* 2000, **150**:489-498.

42. Li X, Gould SJ: **PEX11 promotes peroxisome division independently of peroxisome metabolism**. *J Cell Biol* 2002, **156**:643-651.

43. •• Hoepfner D, van den Berg M, Philippsen P, Tabak HF, Hettema EH: **A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in *Saccharomyces cerevisiae***. *J Cell Biol* 2001, **155**:979-990.

Fluorescence microscopy study on peroxisome morphology and dynamics in living *S. cerevisiae* cells showing that proper partitioning of peroxisomes between mother cell and bud is controlled by directed movement in a Myo2-dependent way, along actin filaments. Furthermore, evidence is presented that Vps1p plays a role in peroxisome fission.

44. Titorenko VI, Chan H, Rachubinski RA: **Fusion of small peroxisomal vesicles *in vitro* reconstructs an early step in the *in vivo* multistep peroxisome assembly pathway of *Yarrowia lipolytica***. *J Cell Biol* 2000, **148**:29-44.

45. Titorenko VI, Rachubinski RA: **Peroxisomal membrane fusion requires two AAA family ATPases, Pex1p and Pex6p**. *J Cell Biol* 2000, **150**:881-886.

46. South ST, Sacksteder KA, Li X, Liu Y, Gould SJ: **Inhibitors of COPI and COPII do not block PEX3-mediated peroxisome synthesis.** *J Cell Biol* 2000, **149**:1345-1359.

47. South ST, Baumgart E, Gould SJ: **Inactivation of the endoplasmic reticulum protein translocation factor, Sec61p, or its homolog, Ssh1p does not affect peroxisome biogenesis.** *Proc Natl Acad Sci USA* 2001 **98**:12027-12031.

48. • Faber KN, Haan GJ, Baerends RJ, Kram AM, Veenhuis M: **Normal peroxisome development from vesicles induced by truncated *Hansenula polymorpha* Pex3p**. *J Biol Chem* 2002, **277**:11026-11033.

Evidence is presented for a role of the endomembrane system in re-assembly of peroxisomes in *H. polymorpha pex3* cells upon re-introduction of the defective gene.

49. Sakai Y, Saiganji A, Yurimoto H, Takabe K, Saiki H, Kato N: **The absence of Pmp47, a putative yeast peroxisomal transporter, causes a defect in transport and folding of a specific matrix enzyme**. *J Cell Biol* 1996 **134**:37-51.

50. Van Dijk R, Lahchev K, Kram AM, van der Klei IJ, Veenhuis M: **Isolation of mutants of *Hansenula polymorpha* defective in the assembly of octameric alcohol oxidase**. *FEMS Yeast Res* 2002, **1**:257-263.