OPINION

Peroxisomal-protein import: is it really that complex?

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Peroxisomal enzymes are synthesized in the cytoplasm and imported post-translationally across the peroxisome membrane. Unlike other organelles with a sealed membrane, peroxisomes can import folded enzymes, and they seem to lack intraperoxisomal chaperones. Here, we propose a mechanistic model for the early steps in peroxisomal-matrix-enzyme import, which might help to explain the unusual features of this process.

Protein import into sealed, membrane-bound compartments poses several challenges. The proteins that are destined for each compartment must be distinguished from other cellular proteins, directed to the organelle surface and translocated across the organelle membrane in a manner that ensures their subsequent activity. A common model serves to explain protein translocation into membrane-sealed compartments such as the endoplasmic reticulum (ER), mitochondrion and chloroplast[1]. Each of these organelles prefers to import unfolded protein substrates, and uses intraorganellar chaperones both to pull the substrates into the organelle and to promote their folding within the lumen. By contrast, peroxisomes (see BOX 1) can import folded proteins and even oligomeric protein complexes[2–4]. Furthermore, most peroxisomes seem to lack intraperoxisomal chaperones that could either promote the folding of unfolded import substrates or help pull proteins across the peroxisome membrane[5]. Here, we propose a new mechanistic hypothesis that might help to explain these unique features of peroxisomal-matrix-enzyme import.

Peroxisomal-matrix-enzyme import

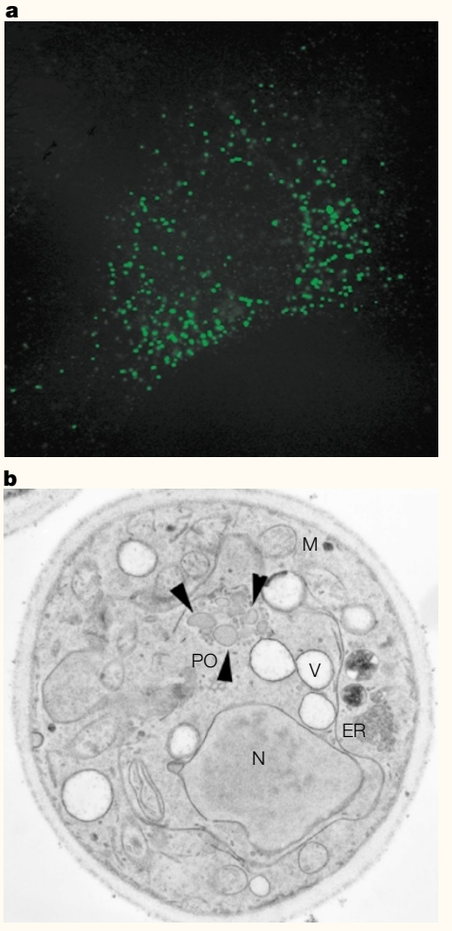
Almost all peroxisomal enzymes have a type-1 peroxisomal targeting signal (PTS1) at their extreme carboxyl terminus[6,7]. The PTS1 typically consists of just three amino acids

Box 1 | **The peroxisome**

Peroxisomes are small organelles (0.1–1 μm in diameter[35]), and are bound by a single, sealed membrane[59]. Although often spherical, they can also form large reticular networks[71]. Peroxisomes are ubiquitous features of both lower eukaryotes, such as yeast, and humans (see figure). In fact, only a few eukaryotes lack peroxisomes, and these unicellular organisms are thought to represent either primitive eukaryotic life forms or descendents of peroxisome-containing eukaryotes[72,73]. Human cells contain ~500 peroxisomes, with the exception of red blood cells, which lack intracellular organelles and have no peroxisomes[35,74].

Peroxisomes are metabolic organelles, and typically contain enzymes that are involved in lipid metabolism, hydrogen peroxide (H_2O_2)-producing oxidases, and the enzyme catalase, which converts H_2O_2 to water and O_2 (REFS 75,76). In yeasts, peroxisomes are the sole site of fatty-acid β-oxidation, and they are required for growth on fatty acids as a sole source for carbon and energy. In humans, peroxisomes contain two fatty-acid β-oxidation pathways and a fatty-acid α-oxidation pathway, which act on signalling lipids (for example, prostanoids, branched-chain fatty acids, dicarboxylic acids and very-long-chain fatty acids) that are only poorly oxidized by mitochondrial fatty-acid β-oxidation pathways. Human peroxisomes also have essential roles in the synthesis of ether-linked lipids and bile acids.

Defects in peroxisomal enzymes are the cause of several human diseases, most of which involve neurological impairment[77,78]. However, the most severe peroxisomal diseases are caused by defects in peroxisome biogenesis, which result in the simultaneous loss of several peroxisomal metabolic functions. These peroxisome biogenesis disorders (PBD) include Zellweger syndrome, and are genetically heterogeneous, with 12 known complementation groups[79]. The genes that are required for peroxisome biogenesis are referred to with the acronym *PEX*, and their products are called peroxins[11]. At present, more than 20 peroxins have been identified in yeast, whereas 15 *PEX* genes are known in humans, including the genes that are defective in 11 of the 12 known complementation groups of PBD patients[79]. Three general phenotypes have been recognized among the known *pex*-deficient cells: lack of peroxisomal-membrane-protein import and peroxisomal-matrix-protein import, resulting in the absence of detectable peroxisomes in the cell; defects in peroxisomal-matrix-protein import but no defect in peroxisome-membrane synthesis or the import of integral peroxisomal membrane proteins; and defects in peroxisome abundance, but no defect in peroxisomal-matrix-protein import or peroxisomal-membrane-protein import[11,79].



The figure shows peroxisomes in human and yeast cells. a | A human cell stained with an antibody to a peroxisomal protein shows the presence of peroxisomes distributed throughout the cytoplasm. b | An electron micrograph of a *Saccharomyces cerevisiae* cell during restoration of peroxisome biogenesis[80] shows a cluster of peroxisomes (PO) and their distribution relative to the vacuoles (V), endoplasmic reticulum (ER), mitochondria (M) and nucleus (N).

Table 1 | **Features of known peroxins**[11,79]

Protein	Characteristics
Peroxins required for peroxisomal-matrix-protein import but not for peroxisomal-membrane-protein import	
PEX1	A large (100–150 kDa) AAA ATPase in yeasts and humans. Interacts with PEX6 and other peroxins. Defects in PEX1 are by far the most common cause of the PBDs (CG1).
PEX2	An ~40-kDa integral PMP with a carboxy-terminal, cytoplasmically exposed zinc RING domain. Has been identified in yeasts and humans, interacts with PEX10 and is defective in CG10 of the PBDs.
PEX4	A small (20–24 kDa) peroxisome-associated ubiquitin-conjugating enzyme that interacts with PEX22. Has been identified in several yeast species, but so far there is no report of PEX4 in any metazoan.
PEX5	An ~70-kDa, predominantly cytoplasmic/partly peroxisomal protein that is found from yeasts to humans. Contains a PTS1-binding domain in its carboxy-terminal, tetratricopeptide-repeat-containing half, interacts with several peroxins (PEX7, PEX8, PEX10, PEX12, PEX13 and PEX14) and is defective in CG2 of the PBDs.
PEX6	An ~100-kDa AAA ATPase found in yeasts and humans. Interacts with PEX1 and is defective in CG4 of the PBDs.
PEX7	An ~40-kDa, WD40-repeat-containing protein that binds the PTS2. Defective in CG11 of the PBDs.
PEX8	A variably sized (60–80 kDa), integral PMP (orphan*) that interacts with PEX5 and is found only in yeast.
PEX9	A 44-kDa integral PMP found only in the yeast *Yarrowia lipolytica*.
PEX10	An ~35-kDa integral PMP with a carboxy-terminal, cytoplasmically exposed zinc RING domain. Has been identified in yeasts and humans, interacts with PEX2, PEX5 and PEX12, and is defective in CG7 of the PBDs.
PEX12	An ~40-kDa integral PMP with a carboxy-terminal, cytoplasmically exposed zinc RING domain. Has been identified in yeasts and humans, interacts with PEX5 and PEX10, and is defective in CG3 of the PBDs.
PEX13	An ~44-kDa integral PMP with a carboxy-terminal, cytoplasmically exposed SH3 domain. Has been identified in yeasts and humans, interacts with PEX5 and PEX14, and is defective in CG13 of the PBDs.
PEX14	An ~40-kDa PMP (orphan) of yeasts and humans that binds to PEX5, PEX13 and PEX17.
PEX15	A 44-kDa integral PMP (orphan) that has been identified only in the yeast *Saccharomyces cerevisiae*.
PEX17	An ~25-kDa integral PMP (orphan) that interacts with PEX14. Has been identified only in *S. cerevisiae*.
PEX18	A 31-kDa soluble protein involved only in PTS2-protein import. It is highly similar to PEX21, and might act as a PEX7 chaperone. Identified only in *S. cerevisiae*.
PEX20	A 46-kDa soluble protein involved only in PTS2-protein import. Identified only in *Y. lipolytica*.
PEX21	A 31-kDa soluble protein involved only in PTS2-protein import, is highly similar to PEX18, and might act as a PEX7 chaperone. Identified only in *S. cerevisiae*.
PEX22	A 20-kDa integral PMP of yeasts that interacts with PEX4.
PEX23	A 46-kDa integral PMP. Identified only in *Y. lipolytica*.
Djp1	A DnaJ-like protein required for normal import of peroxisomal matrix proteins.
Peroxins required for peroxisomal-membrane- and matrix-protein import	
PEX3	An ~40-kDa integral PMP (orphan) in yeast and humans that binds PEX19 and is defective in CG12 of the PBDs.
PEX16	In humans, PEX16 is a 36-kDa integral PMP (orphan) that binds PEX19 and is defective in CG9 of the PBDs.
PEX19	A 33-kDa farnesylated protein (orphan) of yeasts and humans. Predominantly cytoplasmic/partly peroxisomal, binds all known integral PMPs and recognizes many PMP-targeting signals. Is defective in CG14 of the PBDs.
Peroxins that regulate peroxisome abundance but are not required for protein import	
PEX11	An ~25-kDa integral PMP required for normal peroxisome abundance. Many species contain several *PEX11* genes.

CG, complementation group; PBD, peroxisome-biogenesis disorder; PEX, peroxin; PMP, peroxisomal membrane protein; PTS, peroxisome targeting signal; SH3, Src-Homology 3. *Refers to peroxins that are not a member of a known protein family.

(serine–lysine–leucine$_{COOH}$ or a conservative variant[6]), and is sufficient to direct proteins into the peroxisome lumen in protozoans, yeast and mammalian cells[8]. Only a few peroxisomal enzymes use a different targeting signal, the amino-terminally located PTS2 (REF. 9). Import of PTS1 and PTS2 proteins requires around 20 *PEX* genes and their products; the peroxins (TABLE 1).

PTS1 proteins are recognized by PEX5 (REF. 10), whereas PTS2 proteins are recognized by PEX7 (REF. 11). In mammals, the long isoform of PEX5 (PEX5L) is also required for PTS2-protein import, as it binds to PEX7 and is required for PEX7 transit to peroxisomes[12,13]. These observations indicate that PEX5 is the direct or indirect import receptor for all newly synthesized peroxisomal matrix enzymes (nsPMEs) in these species. In yeast, however, PEX5 is not required for PTS2-protein import and only interacts with PEX7 indirectly through linking proteins such as PEX14 (REF. 14). PEX5 is a predominantly cytoplasmic, partly peroxisomal protein that cycles between the cytoplasm and peroxisomes[15], which indicates that peroxisomal-protein import involves a complex interplay of cytoplasmic and peroxisomal events[16] (FIG. 1).

Roles in receptor docking have been proposed for PEX13 and PEX14 — two peroxisomal membrane proteins (PMPs) that interact with PEX5 (REFS 14,17–20). PEX8, PEX10 and PEX12 are a trio of PMPs that also bind to PEX5 but are not required for receptor docking, which indicates that they act subsequently, probably in protein translocation[15,21–23]. As for receptor recycling, it has been proposed[24,25] that a ubiquitin-conjugating enzyme — PEX4 — might be required for this last stage of the PEX5 cycle, and PEX4 would presumably require the involvement of its partner, PEX22 (REF. 26). Other peroxins (TABLE 1) also seem to have important roles in

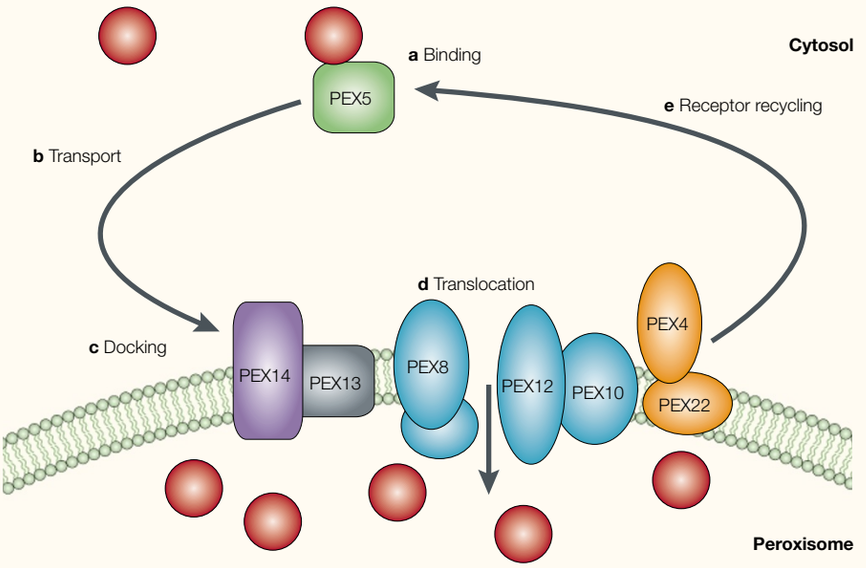


Figure 1 | **A general model of peroxisomal-matrix-enzyme import.** The dynamic distribution of PEX5 indicates that matrix-enzyme import involves: **a** | binding of enzymes (red circles) by the import receptors (shown here as PEX5 in green); **b** | transport of receptor–enzyme complexes to the peroxisome surface; **c** | docking of receptor–enzyme complexes through protein–protein interactions with peroxisomal membrane proteins, such as PEX14 (purple) and, perhaps, PEX13 (grey); **d** | dissociation of receptor–enzyme complexes and enzyme translocation through a proteinaceous pore (perhaps containing PEX8, PEX10 and PEX12, all shown in blue); and **e** | receptor recycling, which might involve PEX4 and PEX22 (both shown in orange). PEX, peroxin.

nsPME import, but less is known about their point of action[11].

This general model of peroxisomal-matrix-enzyme import has been useful for assigning general functions to several of the known peroxins, but suffers from a lack of mechanistic detail. For example, peroxisomal-matrix-enzyme import requires ATP[27,28], and this model makes no predictions about where ATP is consumed or how ATP hydrolysis promotes enzyme translocation. This model also fails to explain how peroxisomes import folded, oligomeric enzymes across a sealed membrane[2–4]. In light of these limitations, we have re-examined the existing data on peroxisomal-matrix-protein import from a mechanistic perspective. The remainder of this article presents a more detailed description of what has arisen from our reconsideration of these data.

The 'preimplex' hypothesis

To gain insight into the molecular mechanisms of matrix-protein import, we concentrated our attention on those aspects of peroxisomal-matrix-protein import for which the biochemical data are most reliable. We began with the recognition of PTS1-containing enzymes by PEX5, the step in import that has been subjected to the most rigorous biochemical analysis. Biochemical and X-ray-crystallography structure data have shown that PEX5 binds one PTS1 per PEX5 monomer, and that PEX5 behaves as an oligomer — most probably a tetramer — with several PTS1-binding sites[10,29]. On its own, the fact that PEX5 is multivalent with respect to its PTS1 ligands is not remarkable. However, the fact that PEX5 can bind several nsPMEs becomes more intriguing when we consider that peroxisomes can import oligomeric peroxisomal enzymes[2–4], and most, if not all, peroxisomal enzymes are oligomers[30–33]. So, the high-affinity interactions between PEX5 and nsPMEs (K_d = ~100 nM)[10] are likely to be mutually multivalent. On the basis of these considerations, we propose that nsPMEs form large nsPME–PEX5 complexes shortly after their synthesis but before their import. We refer to these pre-import complexes of nsPMEs as 'preimplexes'.

"We see preimplex formation as a stochastic process that is controlled primarily by the concentrations of the relevant proteins in the cytoplasm and their affinities for one another."

We see preimplex formation as a stochastic process that is controlled primarily by the concentrations of the relevant proteins in the cytoplasm and their affinities for one another (FIG. 2). In such a system, there are many ways for nsPMEs to interact with preimplexes. For example, peroxisomal enzymes that oligomerize rapidly and/or have a low affinity for PEX5 are more likely to oligomerize before they enter preimplexes, whereas proteins that oligomerize more slowly and/or have a relatively high affinity for PEX5 might enter the preimplex as monomers. Even monomeric proteins should enter preimplexes, provided they contain a PTS1. An intrinsic part of the preimplex hypothesis is that some nsPMEs must oligomerize before, or during, preimplex formation, otherwise the nsPME–PEX5 interactions would not be mutually multivalent. However, various factors (enzyme concentration, degree of enzyme oligomerization and affinity of the PTS1 of each enzyme for PEX5) make it difficult to predict exactly what proportion of peroxisomal enzymes has to oligomerize before, or during, preimplex formation in order for it to proceed.

Another prediction of the preimplex hypothesis is that other PEX5-binding proteins might have a significant effect on preimplex dynamics. For example, PEX14 is particularly suited to having such a role. PEX14 is the primary PEX5-docking site in the peroxisome membrane, and it is a dimeric protein with at least one high-affinity PEX5-binding site per monomer[29,34]. Each PEX5 monomer has several PEX14-binding sites, and so PEX5–PEX14 interactions are also mutually multivalent. In addition to helping tether preimplexes to the peroxisome surface, PEX5–PEX14 interactions might actually promote preimplex expansion by linking otherwise distinct nsPME–PEX5 complexes at PEX14 dimers (FIG. 2).

Although the biochemical properties of peroxisomal enzymes, PEX5 and PEX14 indicate that preimplex assembly is probable, what possible benefit is derived from assembling large nsPME–PEX5 complexes on the peroxisome surface before nsPME translocation? One possibility is that the multivalent nature of nsPME–PEX5–PEX14 interactions allows the rapid and specific delivery of nsPMEs to the peroxisome using only diffusion and the binding energy of these protein–protein interactions. Such a mechanism might explain why PEX5-docking factors are the only proteins that have been implicated in delivering PEX5

to the peroxisome surface[11]. Another advantage is that preimplex formation would generate an extremely high concentration of nsPMEs in the vicinity of the translocation machinery — a situation that is likely to promote their subsequent translocation.

Evidence for the preimplex hypothesis?

In considering the preimplex hypothesis, we wondered whether there might already be some supporting evidence in the literature. Consistent with the cytosol-to-peroxisome import of all peroxisomal proteins[35], peroxisomal enzymes of the yeast *Candida boidinii* are synthesized in the cytoplasm — where they are first detected as monomers — and are subsequently imported into peroxisomes, where they are oligomeric, metabolically functional enzymes[32,33]. However, when Bellion and Goodman[36] carried out a more detailed analysis of peroxisomal-enzyme biogenesis, they observed that several peroxisomal enzymes enter an extremely large, short-lived protein complex immediately after their synthesis but before their translocation into the peroxisome lumen. When these large nsPME-containing complexes were recovered using an antibody to one peroxisomal-matrix enzyme, other unrelated nsPMEs were present in the immunoprecipitates at proportions that approached the ratios of these enzymes in mature peroxisomes[36]. These same enzymes were no longer associated with one another after their import into peroxisomes. Bellion and Goodman[36] also reported that the nsPME-containing complexes could be detected primarily in the cytoplasm but were also present on the peroxisome, and were bound to fragments of the peroxisome membrane. These results are most easily explained by the transit of several nsPMEs through a common complex such as the preimplex; mere association with a large, proteinaceous import apparatus could not explain the simultaneous transit of different nsPMEs through a shared protein complex.

Preimplex disassembly

Mature peroxisomal enzymes do not exist in preimplex-like complexes[31,35,36], so preimplex disassembly must therefore occur before, or during, the translocation process. Once again, Bellion and Goodman[36] have provided an interesting clue to understanding this disassembly process by establishing that the disassembly of the pre-import nsPME-containing complexes is blocked by treatments that deplete cellular levels of ATP. This result indicates that ATPases might be required for preimplex disassembly. Perhaps the most obvious candidates are chaperones of the heat shock protein 70 (Hsp70) family. Hsp70 proteins — as well as their relatives and partners — are necessary for many protein-translocation events, catalyse ATP-dependent protein folding/unfolding reactions and have even been implicated in peroxisomal-protein import[1,5,37–40]. However, it is also possible that the role of Hsp70 proteins in peroxisomal-matrix-protein import is restricted to other, less-specific aspects of peroxisomal-protein import, such as the folding of peroxisomal enzymes before their recognition by the PTS receptors[37] or the folding of peroxins. The AAA family of ATPases, which often act in the disassembly of large protein complexes[41,42], are a second class of ATPases that might be involved in preimplex disassembly. Interestingly, peroxisome biogenesis requires two AAA proteins — PEX1 and PEX6 (REF. 11). PEX1 and PEX6 physically interact with one another, and phenotypic studies indicate that these AAA ATPases are involved in nsPME import[11]. However, no

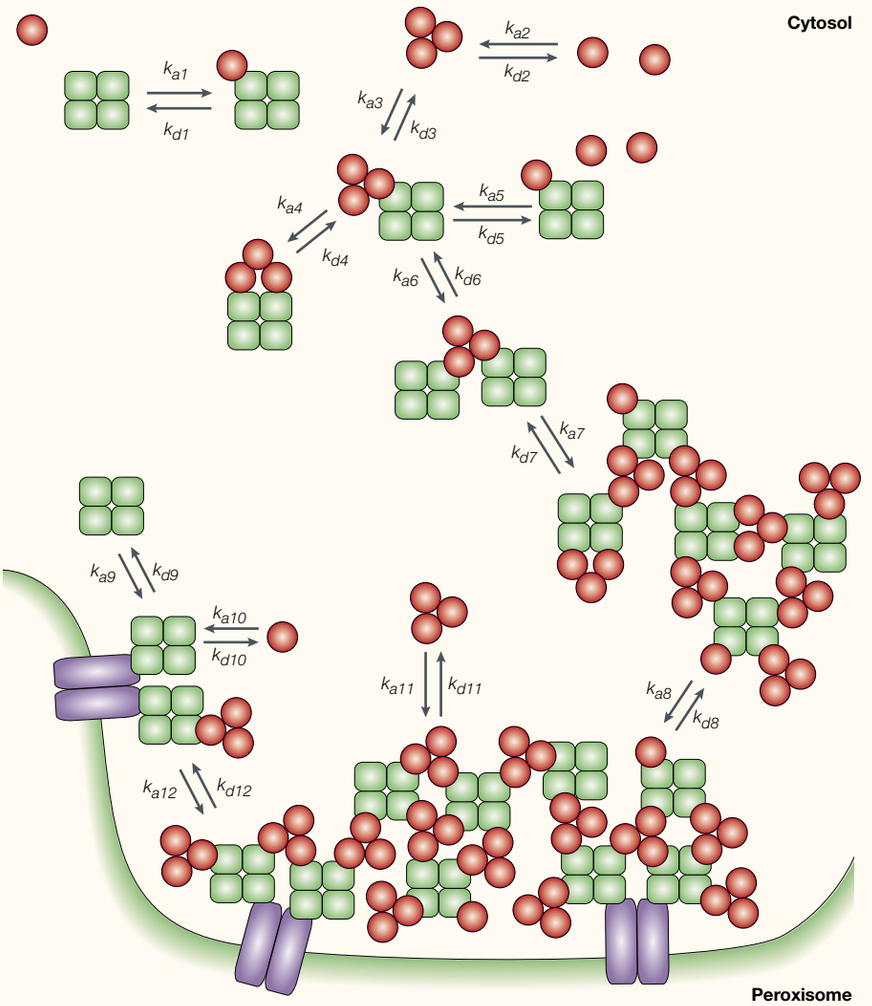


Figure 2 | **Kinetic parameters that affect preimport-complex assembly.** Numerous protein–protein interactions might affect pre-import complex ('preimplex') dynamics — even in a simplified situation in which a single, trimeric protein with a type-1 peroxisomal targeting signal (PTS1; red circles) is the only ligand for tetrameric PEX5 (green squares). Included among these are: (k_1), binding of an enzyme monomer to PEX5; (k_2), enzyme oligomerization; (k_3), binding of an enzyme oligomer to a single PTS1-binding site on PEX5; (k_4), binding of an attached enzyme oligomer to a second PTS1-binding site on PEX5; (k_5), enzyme oligomerization on a PEX5 tetramer; (k_6), binding of PEX5 tetramers to nsPME–PEX5 complexes; (k_7), crosslinking between distinct preimplexes in the cytosol; (k_8), crosslinking between cytoplasmic preimplexes and peroxisome-associated preimplexes; (k_9), PEX5 binding to PEX14 (purple rectangles); binding of enzyme monomers (k_{10}) and oligomers (k_{11}) to membrane-bound PEX5; and (k_{12}), crosslinking between distinct PEX14-linked preimplexes at the peroxisome membrane. *a*, association; *d*, dissociation; nsPME, newly synthesized peroxisomal matrix enzyme; PEX, peroxin.

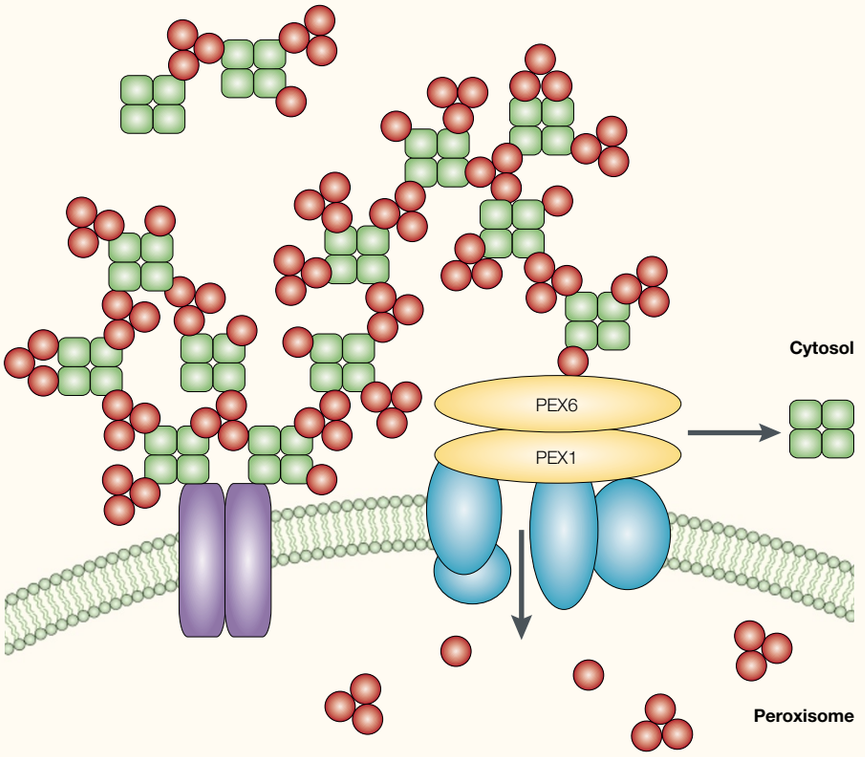


Figure 3 | **A model for PEX1 and PEX6 in peroxisomal enzyme translocation.** A PEX1–PEX6 complex (yellow) binds to components of the translocation apparatus (blue) and to preimplexes (red and green) that are attached to the peroxisome by PEX14 (purple rectangles). PEX1 and PEX6 use ATP hydrolysis to disassemble preimplexes, assemble the translocation pore and direct the vectorial movement of enzyme into the peroxisome lumen. These events might occur sequentially or synchronously. Products of preimplex disassembly could be individual polypeptides or some type of nsPME–PEX5 subcomplex that is much smaller than the preimplex, although the final product of the entire reaction is intraperoxisomal enzyme and cytoplasmic PEX5. Although the PEX5 being released is shown as a tetramer, the translocation process might actually generate a different product, such as free, monomeric PEX5. nsPME, newly synthesized peroxisomal matrix enzyme; PEX, peroxin.

hypothesis has been put forward to explain their role in this process. We propose that PEX1 and PEX6 facilitate preimplex disassembly and participate in other processes, such as assembly of the translocation apparatus and the vectorial transport of proteins across the membrane.

One of the first predictions of this hypothesis is that defects in PEX1 and/or PEX6 should result in preimplex accumulation on the outer surface of the peroxisome, at least to the stoichiometric limits of PEX5 and PEX14, or any other factor that has a limiting role in preimplex assembly. Consistent with this prediction, human cells that lack *PEX1* or *PEX6* contain far more particulate peroxisomal matrix enzymes than cells that lack most other *PEX* genes[43–45], and these enzyme particulates are not in the peroxisome lumen but are on the outside of peroxisomes and seem to be surrounded by empty, doubled-over peroxisomal membranes[45] (C. S. C. and S. J. G., unpublished observations). Further supporting evidence comes from the observation that *Pichia pastoris pex1* and *pex6* mutants have large particles that contain peroxisomal enzymes, PEX5 and fragments of peroxisome membranes[46,47].

Preimplex dissociation

Continual preimplex assembly and disassembly would represent futile cycles of ATP hydrolysis. Therefore, we propose that preimplex disassembly will be tightly coupled to enzyme translocation. Tight coupling between these processes would allow nsPMEs to be transferred from the preimplex into the translocation apparatus while the nsPMEs are still bound to PEX5, which prevents a leak of these enzymes back to the cytoplasm and removes the need for more enzyme-binding proteins. In addition, tight coupling between preimplex disassembly and subsequent steps in import is an attractive proposal, because it would place PEX1 and PEX6 in a good position to provide the energy that is needed for enzyme translocation. Although there is no evidence that PEX1 or PEX6 catalyse enzyme translocation, it is intriguing that a recent two-hybrid study of *Saccharomyces cerevisiae* peroxins showed potential interactions between PEX1, PEX6 and several factors that are implicated in enzyme translocation (PEX5, PEX8, PEX10 and PEX12) (Y. Liu, K. Sacksteder, J. C. Morrell and S. J. G., unpublished observations). In addition, it has recently been shown that Cdc48/VCP — the AAA ATPase that is most closely related to PEX1 and PEX6 — is required for the retro-translocation of proteins from the ER lumen to the cytoplasm[48–50]. Physical coupling of preimplex dissociation and enzyme translocation could be represented by PEX1 and PEX6 sitting at the top of the translocation apparatus, mediating the transfer of nsPMEs from the preimplex into the cavity of the translocon (FIG. 3). This particular scheme also allows PEX1, PEX6 and, perhaps, even PEX5 to cap the translocon and maintain a sealed peroxisome membrane. This hypothesis is also consistent with the results of a recent epistasis analysis, which indicated that virtually all peroxins are required for normal PEX1 and PEX6 function[25].

The observations of oligomeric protein import and 'piggyback' protein import[2–4,51] — in which proteins that lack a PTS are imported as long as they bind to a PTS-containing protein in the cytoplasm — raise the possibility that the translocation pore in the peroxisome membrane might be extremely large. This notion is also reinforced by the observation that 4–9-nm gold particles that are coated with PTS1 peptides can be imported into peroxisomes *in vivo*[4]. Some have even proposed[52,53] that peroxisomal-protein translocation might occur through large pores that are similar to those in the nuclear envelope, rather than the smaller, gated pores of the ER protein

"Another advantage of the preimplex hypothesis is that it will be relatively easy to test. Several predictions can be deduced from the preimplex hypothesis and many of these are amenable to direct analysis."

"The preimplex hypothesis offers explanations for the ATP dependence of peroxisomal-enzyme import, how peroxisomes import folded enzymes, and why peroxisomes do not require lumenal chaperones."

translocon[54,55]. Although this might be true, the data so far argue only for the import of oligomers of ~450 kDa[56] or smaller[2–4,57], and the actual translocation substrates might be considerably smaller, depending on the products and timing of preimplex disassembly. For example, preimplex disassembly might convert oligomeric enzymes to monomers before the actual translocation event. Furthermore, a peroxisomal-protein translocation pore with just 2–3 times the diameter of the ER protein translocon (5 nm in the open state) could explain the passage of fully folded proteins, moderately sized oligomers and even 4–9-nm gold particles. The upper size limit on what can pass across the peroxisome membrane has not been established, but it is interesting to note that the peroxisomal enzyme alcohol oxidase — which assembles into octamers of approximately 500 kDa — seems to oligomerize only after its association with peroxisomes[58]. It should also be noted that the peroxisome membrane is impermeable to small metabolites and protons[59,60], and that freeze-fracture electron-microscopic analysis failed to show any large pores in the peroxisome membrane[61–63].

Preimplex hypothesis and other models

Several reviews[53,64] have proposed that an important debate in the peroxisome field is between an 'extended-shuttle' mechanism of matrix-enzyme import, in which PEX5 is translocated freely into the peroxisome lumen and exported back to the cytoplasm, and a 'simple-shuttle' mechanism, in which PEX5 only brings nsPMEs to the peroxisome surface. However, the 'simple-shuttle' mechanism precludes a role for PEX5 in the transmembrane translocation process, demands that PEX5 dissociates from its cargo of PTS1 proteins before the enzyme translocation event, and requires the existence of some factor(s) other than PEX5 to bind and carry the PTS1 proteins across the membrane during the translocation event[53]. As such, several observations argue against the 'simple-shuttle' model, including: the fact that small amounts of PEX5 are embedded in the peroxisome membrane[21,65], and are present in the peroxisome lumen[66]; the direct, physical interactions between PEX5 and several putative protein translocation factors (PEX8, PEX10 and PEX12; REFS 21–23); and the inability to find proteins other than PEX5 and PEX7 in searches for peroxisomal-enzyme-binding proteins[67] (S. J. G., unpublished observations). Therefore, we feel that a more relevant debate is between the 'extended-shuttle' model and a 'trap-door' model of protein translocation (FIG. 4). In the 'trap-door' model, a type of small nsPME–PEX5 complex would be inserted into the translocation apparatus with a topology that delivers the enzymes into the peroxisome environment. A reduction in the affinity of PEX5 for its PTS1 cargo would complete the enzyme translocation event, and the release of unoccupied PEX5 to the cytoplasm would complete the PEX5 cycle. This model differs significantly from the 'extended-shuttle', in that PEX5 is not translocated and released into the peroxisome lumen, although it might be exposed to the chemical and enzymatic environment of the peroxisome lumen during the translocation process. It should be noted that the preimplex hypothesis is consistent with both the 'extended-shuttle' and 'trap-door' models of peroxisomal-matrix-enzyme import.

Impact of the preimplex hypothesis

We have already discussed how the preimplex hypothesis can explain the ATP requirement of peroxisomal-matrix-enzyme import[27,28,36], the rapid and specific delivery of nsPMEs to the peroxisome[35,68], the import of folded proteins and oligomeric enzymes[2–4], the various phenotypes of *pex1* and *pex6* mutants[11,25,45], and the interactions between PEX1 and PEX6 and several putative translocation factors[11] (Y. Liu, K. Sacksteder, J. C. Morrell, and S. J. G., unpublished observations). However, the preimplex hypothesis might also help us understand several other observations. For example, the preimplex hypothesis proposes that PEX14 serves mainly to nucleate and localize preimplexes to the peroxisome membrane, which might explain why PEX5 overexpression can suppress a *pex14*-null mutant, and why small amounts of peroxisomal matrix enzymes are imported into peroxisomes even in the absence of PEX14 (REF. 69). The preimplex hypothesis also predicts that any protein that enters the preimplex will be delivered into the translocation apparatus, which might explain the lack of selectivity that is apparent in the many instances of 'piggyback' protein import[2,3,51]. In addition, the preimplex hypothesis predicts that PEX5 carries nsPMEs into the translocation apparatus, and so is a crucial component of the

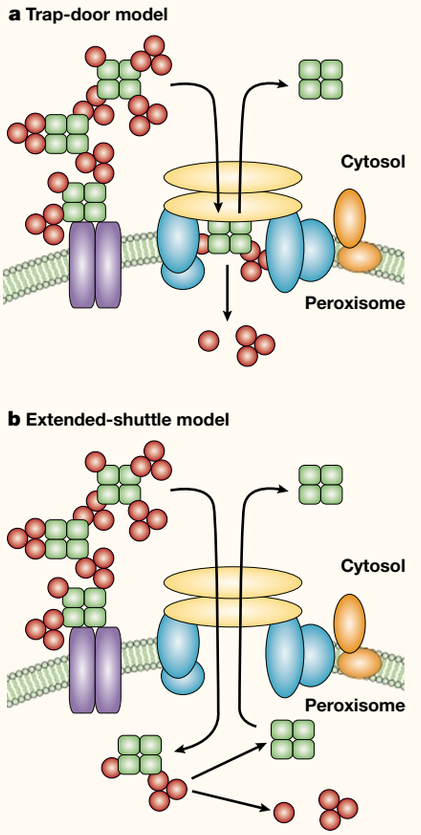


Figure 4 | **The 'trap-door' and 'extended-shuttle' models of enzyme translocation.** **a** | In the trap-door model, enzymes (red circles) are delivered to the peroxisome lumen while they are still bound to PEX5 (green squares). Complete translocation is achieved by lowering the affinity of PEX5 for the PTS1. This model proposes that PEX5 is an integral component of the translocation apparatus (blue and yellow), but that it is not released into the peroxisome lumen, except as a consequence of aberrant events. **b** | In the extended-shuttle model, nsPME–PEX5 complexes (red circles and green squares) are translocated completely into the peroxisome lumen, followed by nsPME–PEX5 dissociation within the peroxisome lumen and retro-translocation of free PEX5 back to the cytoplasm, presumably through the same translocation apparatus (blue and yellow). PEX14 is represented by purple rectangles, and PEX4 and PEX22 are shown in orange. nsPME, newly synthesized peroxisomal matrix enzyme; PEX, peroxin.

Box 2 | **Predictions that can be deduced from the preimplex hypothesis**

- Newly synthesized peroxisomal matrix enzymes (nsPMEs) should enter common, large peroxin (PEX)5-containing preimplexes shortly after their synthesis and exit from these complexes before, or during, their translocation.
- Preimplex formation should require oligomeric PEX5.
- Preimplex formation will require the expression of oligomeric peroxisomal-matrix enzymes.
- In human cells, where PEX5L binds PEX7 and is required for peroxisomal targeting signal (PTS)2-protein import, PTS2 proteins should also enter preimplexes, and this entry should be dependent on PEX5L and PEX7.
- In yeast, entry of PTS2 proteins into preimplexes might require PEX7-linking proteins, such as PEX18 and PEX21 (REF. 70), as well as PEX7.
- Preimplex dynamics will be modulated by PEX14, the primary PEX5-docking site in the peroxisome membrane. Other PEX5-binding proteins might also affect preimplex dynamics.
- Preimplex disassembly will be dependent on PEX1, PEX6 and ATP, and involve interactions between PEX5 and the disassembly apparatus.
- Cells that lack PEX1 or PEX6 should accumulate nsPME–PEX5 complexes on the outer surface of peroxisomes. Due to the multivalent nature of preimplex–PEX14 interactions, this should cause a zippering effect that leads to partial wrapping of preimplexes by the empty peroxisome membrane.
- Preimplex disassembly will require one or more PEX1/PEX6-interacting proteins in the peroxisome membrane (perhaps PEX8, PEX10 or PEX12).
- Preimplex disassembly will be tightly coupled to translocon assembly and enzyme translocation.
- Ubiquitin-mediated destruction of PEX5 will be sensitive to preimplex assembly and disassembly.

translocation machine, which might explain why PEX5 interacts with putative protein-translocation components[21–23].

Finally, the protein-import model we propose here might be relevant to an obscure observation. It is now well established that PEX5 is rapidly degraded in yeast or human cells that lack either PEX1 or PEX6 (REFS 15,25). If, as we propose, PEX1 and PEX6 do act in peroxisomal-matrix-enzyme import by disassembling preimplexes and promoting the subsequent steps in matrix-enzyme translocation, it might be useful to view PEX5 destruction as a quality-control mechanism that serves to eliminate 'inappropriate' nsPME–PEX5 complexes. If this is so, the defect in preimplex disassembly that is caused by loss of PEX1 or PEX6 might increase the portion of PEX5 in such 'inappropriate' complexes, and hence increase the rate of PEX5 destruction. Furthermore, epistasis analysis in the yeast *Pichia pastoris* has shown that nearly all peroxins are required for the accelerated rate of PEX5 destruction that is seen in *pex1* and *pex6* mutants[25]. This observation supports our present hypothesis that several matrix-protein import factors are required for PEX1 and PEX6 to carry out their functions in peroxisomal-matrix-enzyme import.

Testing the preimplex hypothesis

Another advantage of the preimplex hypothesis is that it will be relatively easy to test. Several predictions can be deduced from the preimplex hypothesis and many of these are amenable to direct analysis, a few of which are listed (BOX 2).

Concluding remarks

The preimplex hypothesis offers explanations for the ATP dependence of peroxisomal-enzyme import, how peroxisomes import folded enzymes, and why peroxisomes do not require lumenal chaperones. It also makes clear predictions about the general roles of PEX1, PEX5, PEX6 and PEX14 in the earliest stages of nsPME import. As such, the preimplex hypothesis holds the promise of advancing our understanding of a molecular mechanism of peroxisomal-matrix-enzyme import, and might be a stepping stone to an accurate, mechanistic model of this process. At the same time, it should be noted that the preimplex hypothesis does not represent a comprehensive model for peroxisomal-matrix-protein import. The mechanism of receptor recycling, the roles of PEX1 and PEX6 in other aspects steps of import, and the positive role of ubiquitin and the ubiquitin-conjugating enzyme PEX4 remain to be addressed, as does the role of most factors that are required for peroxisomal-matrix-enzyme import. It is our hope that a mechanistic understanding of the translocation steps in matrix-enzyme import will lead to more detailed, mechanistic models to explain the later stages of this process.

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1. Agarraberes, F. A. & Dice, J. F. Protein translocation across membranes. *Biochim. Biophys. Acta* **1513**, 1–24 (2001).
2. McNew, J. A. & Goodman, J. M. An oligomeric protein is imported into peroxisomes *in vivo*. *J. Cell Biol.* **127**, 1245–1257 (1994).
3. Glover, J. R., Andrews, D. W. & Rachubinski, R. A. *Saccharomyces cerevisiae* peroxisomal thiolase is imported as a dimer. *Proc. Natl Acad. Sci. USA* **91**, 10541–10545 (1994).
4. Walton, P., Hill, P. & Subramani, S. Import of stably folded proteins into peroxisomes. *Mol. Biol. Cell* **6**, 675–683 (1995).
5. Walton, P. *et al.* Involvement of 70 kD heat shock proteins in peroxisomal import. *J. Cell Biol.* **125**, 1037–1046 (1994).
6. Gould, S. J. *et al.* A conserved tripeptide sorts proteins to peroxisomes. *J. Cell Biol.* **108**, 1657–1664 (1989).
7. Subramani, S. Protein import into peroxisomes and biogenesis of the organelle. *Annu. Rev. Cell Biol.* **9**, 445–478 (1993).
8. Gould, S. J. *et al.* Peroxisomal protein import is conserved between yeast, plants, insects and mammals. *EMBO J.* **9**, 85–90 (1990).
9. Swinkels, B. W. *et al.* A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. *EMBO J.* **10**, 3244–3262 (1991).
10. Gatto, G. J. Jr. *et al.* Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5. *Nature Struct. Biol.* **7**, 1091–1095 (2000).
11. Sacksteder, K. A. & Gould, S. J. The genetics of peroxisome biogenesis. *Annu. Rev. Genet.* **34**, 623–652 (2000).
12. Otera, H. *et al.* 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 (2000).
13. Dodt, G. *et al.* Domain mapping of human PEX5 reveals functional and structural similarities to *S. cerevisiae* Pex18p and Pex21p. *J. Biol. Chem.* **276**, 41769–41781 (2001).
14. Girzalsky, W. *et al.* Involvement of Pex13p in Pex14p localization and peroxisomal targeting signal 2-dependent protein import into peroxisomes. *J. Cell Biol.* **144**, 1151–1162 (1999).
15. Dodt, G. & Gould, S. J. 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.* **135**, 1763–1774 (1996).
16. Gould, S. J. & Valle, D. The genetics and cell biology of the peroxisome biogenesis disorders. *Trends Genet.* **16**, 340–344 (2000).
17. Albertini, M. *et al.* Pex14p, a peroxisomal membrane protein binding both receptors of the two PTS-dependent import pathways. *Cell* **89**, 83–92 (1997).
18. Gould, S. J. *et al.* PEX13p is an SH3 protein in the peroxisome membrane and a docking factor for the PTS1 receptor. *J. Cell Biol.* **135**, 85–95 (1996).
19. Elgersma, Y. *et al.* 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 (1996).
20. Erdmann, R. & Blobel, G. Identification of Pex13p, a peroxisomal membrane receptor for the PTS1 recognition factor. *J. Cell Biol.* **135**, 111–121 (1996).
21. Chang, C. C. *et al.* PEX12 binds PEX5 and PEX10 and acts downstream of receptor docking in peroxisomal matrix protein import. *J. Cell Biol.* **147**, 761–773 (1999).
22. Rehling, P. *et al.* Pex8p, an intraperoxisomal peroxin of *Saccharomyces cerevisiae* required for protein transport into peroxisomes binds the PTS1 receptor pex5p. *J. Biol. Chem.* **275**, 3593–3602 (2000).
23. Okumoto, K., Abe, I. & Fujiki, Y. Molecular anatomy of the peroxin Pex12p: RING finger domain is essential for the Pex12p function and interacts with the peroxisome targeting signal type 1-receptor Pex5p and a RING peroxin, Pex10p. *J. Biol. Chem.* **275**, 25700–25710 (2000).
24. van der Klei, I. J. *et al.* The ubiquitin-conjugating enzyme

Pex4p of *Hansenula polymorpha* is required for efficient functioning of the PTS1 import machinery. *EMBO J.* **17**, 3608–3618 (1998).
25. Collins, C. S. *et al.* The peroxisome biogenesis factors Pex4p, Pex22p, Pex1p, and Pex6p act in the terminal steps of peroxisomal matrix protein import. *Mol. Cell. Biol.* **20**, 7516–7526 (2000).
26. Koller, A. *et al.* Pex22p of *Pichia pastoris*, essential for peroxisomal matrix protein import, anchors the ubiquitin-conjugating enzyme, Pex4p, on the peroxisomal membrane. *J. Cell Biol.* **146**, 99–112 (1999).
27. Imanaka, T., Small, G. M. & Lazarow, P. B. Translocation of acyl-CoA oxidase into peroxisomes requires ATP hydrolysis but not a membrane potential. *J. Cell Biol.* **105**, 2915–2922 (1987).
28. Wendland, M. & Subramani, S. Cytosol-dependent peroxisomal protein import in a permeabilized cell system. *J. Cell Biol.* **120**, 675–685 (1993).
29. Schliebs, W. *et al.* Recombinant human peroxisomal targeting signal receptor PEX5. Structural basis for interaction of PEX5 with PEX14. *J. Biol. Chem.* **274**, 5666–5673 (1999).
30. Tolbert, N. E. Metabolic pathways in peroxisomes and glyoxysomes. *Annu. Rev. Biochem.* **50**, 133–157 (1981).
31. Hashimoto, T. Peroxisomal β-oxidation enzymes. *Cell Biochem. Biophys.* **32**, 63–72 (2000).
32. Goodman, J. M. Dihydroxyacetone synthase is an abundant constituent of the methanol-induced peroxisome of *Candida boidinii*. *J. Biol. Chem.* **260**, 7108–7113 (1985).
33. Goodman, J. M. *et al.* Alcohol oxidase assembles post-translationally into the peroxisome of *Candida boidinii*. *J. Biol. Chem.* **259**, 8485–8493 (1984).
34. Saidowsky, J. *et al.* 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.* **3**, 34524–34529 (2001).
35. Lazarow, P. B. & Fujiki, Y. Biogenesis of peroxisomes. *Annu. Rev. Cell Biol.* **1**, 489–530 (1985).
36. Bellion, E. & Goodman, J. M. Proton ionophores prevent assembly of a peroxisomal protein. *Cell* **48**, 165–173 (1987).
37. Harano, T. *et al.* Hsp70 regulates the interaction between the peroxisome targeting signal type 1 (PTS1)-receptor Pex5p and PTS1. *Biochem. J.* **357**, 157–165 (2001).
38. Legakis, J. E. & Terlecky, S. R. PTS2 protein import into mammalian peroxisomes. *Traffic* **2**, 252–260 (2001).
39. Hettema, E. H. *et al.* The cytosolic DnaJ-like protein djp1p is involved specifically in peroxisomal protein import. *J. Cell Biol.* **142**, 421–434 (1998).
40. Matouschek, A., Pfanner, N. & Voos, W. Protein unfolding by mitochondria. The Hsp70 import motor. *EMBO Rep.* **1**, 404–410 (2000).
41. Neuwald, A. F. *et al.* AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome Res.* **9**, 27–43 (1999).
42. Vale, R. D. AAA proteins. Lords of the ring. *J. Cell Biol.* **150**, F13–F19 (2000).
43. Yahraus, T. *et al.* The peroxisome biogenesis disorder group 4 gene, *PXAAA1*, encodes a cytoplasmic ATPase required for stability of the PTS1 receptor. *EMBO J.* **15**, 2914–2923 (1996).
44. Reuber, B. E. *et al.* Mutations in *PEX1* are the most common cause of the peroxisome biogenesis disorders. *Nature Genet.* **17**, 445–448 (1997).
45. Hashiguchi, N. *et al.* Peroxisomes are formed from complex membrane structures in PEX6-deficient CHO cells upon genetic complementation. *Mol. Biol. Cell* **13**, 711–722 (2002).
46. Spong, A. P. & Subramani, S. Cloning and characterization of *PAS5*: a gene required for peroxisome biogenesis in the methylotrophic yeast *Pichia pastoris*. *J. Cell Biol.* **123**, 535–548 (1993).
47. Heyman, J. A., Mononsov, E. & Subramani, S. Role of the *PAS1* gene of *Pichia pastoris* in peroxisome biogenesis. *J. Cell Biol.* **127**, 1259–1273 (1994).
48. Rabinovich, E. *et al.* AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol. Cell. Biol.* **22**, 626–634 (2002).
49. Ye, Y., Meyer, H. H. & Rapoport, T. A. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* **414**, 652–656 (2001).
50. Hitchcock, A. L. *et al.* The conserved npl4 protein complex mediates proteasome-dependent membrane-bound transcription factor activation. *Mol. Biol. Cell* **12**, 3226–3241 (2001).
51. Cohen, G. B. *et al.* The human thioesterase II protein binds to a site on HIV-1 Nef critical for CD4 down-regulation. *J. Biol. Chem.* **275**, 23097–23105 (2000).
52. Dammai, V. & Subramani, S. The human peroxisomal targeting signal receptor, Pex5p, is translocated into the peroxisomal matrix and recycled to the cytosol. *Cell* **105**, 187–196 (2001).
53. Smith, M. D. & Schnell, D. J. Peroxisomal protein import: the paradigm shifts. *Cell* **105**, 293–296 (2001).
54. Matlack, K. E., Mothes, W. & Rapoport, T. A. Protein translocation: tunnel vision. *Cell* **92**, 381–390 (1998).
55. Johnson, A. E. & Van Waes, M. A. The translocon: a dynamic gateway at the ER membrane. *Annu. Rev. Cell Dev. Biol.* **15**, 799–842 (1999).
56. Titorenko, V. I. *et al.* Acyl-CoA oxidase is imported as a heteropentameric, cofactor-containing complex into peroxisomes of *Yarrowia lipolytica*. *J. Cell Biol.* **156**, 481–494 (2002).
57. Middelkoop, E. *et al.* Topology of catalase assembly in human skin fibroblasts. *Biochim. Biophys. Acta* **1220**, 15–20 (1993).
58. Stewart, M. Q. *et al.* Alcohol oxidase and dihydroxyacetone synthase, the abundant peroxisomal proteins of methylotrophic yeasts, assemble in different cellular compartments. *J. Cell Sci.* **114**, 2863–2868 (2001).
59. Dansen, T. B. *et al.* Peroxisomes in human fibroblasts have a basic pH. *Nature Cell Biol.* **2**, 51–53 (2000).
60. Van Roermund, C. W. *et al.* The membrane of peroxisomes in *Saccharomyces cerevisiae* is impermeable to NAD(H) and acetyl-CoA under *in vivo* conditions. *EMBO J.* **14**, 3480–3486 (1995).
61. Furuta, K. *et al.* Three-dimensional ultrastructure of normal rat hepatocytes by quick-freezing and deep-etching method. *J. Gastroenterol. Hepatol.* **7**, 486–490 (1992).
62. Kryvi, H., Kvannes, J. & Flatmark, T. Freeze-fracture study of rat liver peroxisomes: evidence for an induction of intramembrane particles by agents stimulating peroxisomal proliferation. *Eur. J. Cell Biol.* **53**, 227–233 (1990).
63. Zaar, K. & Fahimi, H. D. A freeze-etch study of angular marginal-plate-containing peroxisomes in the proximal tubules of bovine kidney. *Cell Tissue Res.* **260**, 409–414 (1990).
64. Rachubinski, R. A. & Subramani, S. How proteins penetrate peroxisomes. *Cell* **83**, 525–528 (1995).
65. Gouveia, A. M. *et al.* Characterization of peroxisomal Pex5p from rat liver. Pex5p in the Pex5p–Pex14p membrane complex is a transmembrane protein. *J. Biol. Chem.* **275**, 32444–32451 (2000).
66. Van der Klei, I. J. *et al.* The *Hansenula polymorpha PER3* gene is essential for the import of PTS1 proteins into the peroxisome matrix. *J. Biol. Chem.* **270**, 17229–17236 (1995).
67. Fransen, M. *et al.* Identification and characterization of the putative human peroxisomal C-terminal targeting signal import receptor. *J. Biol. Chem.* **270**, 7731–7736 (1995).
68. Lazarow, P. B. *et al.* Biogenesis of peroxisomal protein *in vivo* and *in vitro*. *Annal. N. Y. Acad. Sci.* **386**, 285–300 (1982).
69. Salomons, F. A. *et al.* Overproduction of Pex5p stimulates import of alcohol oxidase and dihydroxyacetone synthase in a *Hansenula polymorpha Pex14* null mutant. *J. Biol. Chem.* **275**, 12603–12611 (2000).
70. Purdue, P. E. & Lazarow, P. B. Peroxisome biogenesis. *Annu. Rev. Cell Dev. Biol.* **17**, 701–752 (2001).
71. Schrader, M. *et al.* Real time imaging reveals a peroxisomal reticulum in living cells. *J. Cell Sci.* **113**, 3663–3671 (2000).
72. Muller, M. Peroxisomes of protozoa. *Ann. N. Y. Acad. Sci.* **168**, 291–301 (1969).
73. Adam, R. D. Biology of *Giardia lamblia*. *Clin. Microbiol. Rev.* **14**, 447–475 (2001).
74. Chang, C. C. *et al.* Metabolic control of peroxisome abundance. *J. Cell Sci.* **112**, 1579–1590 (1999).
75. Van den Bosch, H. *et al.* Biochemistry of peroxisomes. *Annu. Rev. Biochem.* **61**, 157–197 (1992).
76. Wanders, R. J. *et al.* Peroxisomal fatty acid α- and β-oxidation in humans: enzymology, peroxisomal metabolite transporters and peroxisomal diseases. *Biochem. Soc. Trans.* **29**, 250–267 (2001).
77. Wanders, R. J. A., Barth, P. G. & Heymans, H. S. A. in *The Metabolic and Molecular Bases of Inherited Disease*, Vol. 2 (eds Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D.) 3219–3256 (McGraw–Hill, New York, 2001).
78. Wanders, R. J. A., Jakobs, C. & Skejldal, O. H. in *The Metabolic and Molecular Bases of Inherited Disease* Vol. 2 (eds Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D.) 3303–3322 (McGraw–Hill, New York, 2001).
79. Gould, S. G., Valle, D. & Raymond, G. V. in *The Metabolic and Molecular Bases of Inherited Disease*, Vol. 2 (eds Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D.) 3181–3217 (McGraw–Hill, New York, 2001).
80. South, S. T., Baumgart, E. & Gould, S. J. Inactivation of the endoplasmic reticulum protein translocation factor, Sec61p, or its homolog, Ssh1p, does not affect peroxisome biogenesis. *Proc. Natl Acad. Sci. USA* **98**, 12027–12031 (2001).

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