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Invited Review

Peroxisome biogenesis and the role of protein import

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Abstract

Peroxisomes are metabolic organelles with enzymatic content that are found in virtually all cells and are involved in β-oxidation of fatty acids, hydrogen peroxide-based respiration and defence against oxidative stress. The steps of their biogenesis involves "peroxins", proteins encoded by PEX genes. Peroxins are involved in three key stages of peroxisome development: (1) import of peroxisomal membrane proteins; (2) import of peroxisomal matrix proteins and (3) peroxisome proliferation. Of these three areas, peroxisomal matrix-protein import is by far the best understood and accounts for most of the available published data on peroxisome biogenesis. Defects in peroxisome biogenesis result in peroxisome biogenesis disorders (PBDs), which although rare, have no known cure to-date. This review explores current understanding of each key area in peroxisome biogenesis, paying particular attention to the role of protein import.

Keywords: peroxisome biogenesis - peroxin - protein import - proliferation

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Introduction

Peroxisomes are metabolic organelles found in virtually all eukaryotic cells. Although the morphology of peroxisomes differs between tissues and species, they are typically between 0.1-1μm in diameter and are surrounded by a single lipid bilayer. They are mostly spherical organelles, although have been shown to form large reticular networks [1, 2]. Three conserved functions of peroxisomes are generally found amongst evolutionary diverse organisms. These are β-oxidation of fatty acids, hydrogen peroxide-based respiration and defence against oxidative stresses. Other specialised roles depend on the organism and cell type.

Plant peroxisomes can be classified into three main types: glyoxysomes, leaf peroxisomes and unspecialised peroxisomes. Glyoxysomes are mainly found in seedlings where the glyoxylate cycle, a modified form of the tricarboxylic acid cycle in plants and yeasts, takes place. The glyoxylate cycle also occurs in certain bacteria, but being prokaryotes, they lack specialised organelles such as peroxisomes that host such reactions. Leaf peroxisomes, as their name suggests, are found in leaf tissues and are the site for part of the glycolate and glycerate pathways of photorespiration. Plant peroxisomes are also implicated in the generation of a range of important hormones and other signalling molecules, including indole acetic acid [3], jasmonate [4], nitric oxide and several reactive oxygen species [5].

In mammals, peroxisomes contain two fatty acid β-oxidation pathways and a fatty acid α-oxidation pathway which act on a specific set of lipids that are weakly oxidised by mitochondrial fatty-acid β-oxidation pathways. These lipids include very long straight chain fatty acids, long chain dicarboxylic acids, branched-chain fatty acids (e.g. phytanic acid is a-oxidised to pristanic acid which is then b-oxidised), di- and trihydroxycoprostanic acid acids and prostanoids [6]. In addition peroxisomes are also thought to be essential for cholesterol biosynthesis [7]. The cholesterol biosynthesis pathway was previously assumed to be localised in the cytosol and endoplasmic reticulum (ER), although it is now thought that most of the enzymes involved in cholesterol biosynthesis exist almost exclusively in the peroxisome [8]. However, it should be noted that it was found in liver cells of *pex5*-/- knockout mice (which lack functional peroxisomes) that putative peroxisomal enzymes were at least as active here as in control mice, hence the mislocalisation of enzymes to the cytosol does not necessarily lead to decreased activity or degradation [9].

In yeasts, peroxisomes are the sole site of fatty acid β-oxidation. Other pathways, which involve the peroxisome include the biosynthesis of lysine and the degradation of methanol and amino acids.

The importance of the peroxisome (and related metabolic processes) is shown by disorders associated with peroxisomal defects. In humans, genetically determined peroxisomal disorders can be divided into two categories: (i) disorders resulting from a defect in a single peroxisomal enzyme and (ii) disorders that result from a deficiency in the biogenesis of the peroxisome, referred to as peroxisome biogenesis disorders (PBDs). The former includes X-linked adrenoleukodystrophy (the most common peroxisomal disorder) whilst the latter includes the various complementation groups of Zellweger's syndrome (a spectrum of diseases of varying severity depending upon the specific allele or the mutant gene).

In recent years studies have produced a wealth of information regarding the proteins required for peroxisome biogenesis, which are referred to as peroxins. To date 27 genes encoding peroxins (*PEX* genes) have been cloned whose products have been found to be involved in matrix protein import, membrane biogenesis and organelle proliferation. This review will examine the current knowledge regarding these three key areas, paying particular attention to the mechanisms involved in protein import, which at the moment are best understood.

Protein import into peroxisomes: matrix proteins

Matrix protein targeting to the peroxisome

Fig. 1 summarises in diagrammatic form the current view of matrix protein import. Peroxisomal matrix proteins are synthesised on free polyribosomes in the cytosol [10] and are imported into the organelle via one of either two pathways requiring two evolutionary conserved peroxisomal targeting signal (PTS) sequences. The most common of these tar-

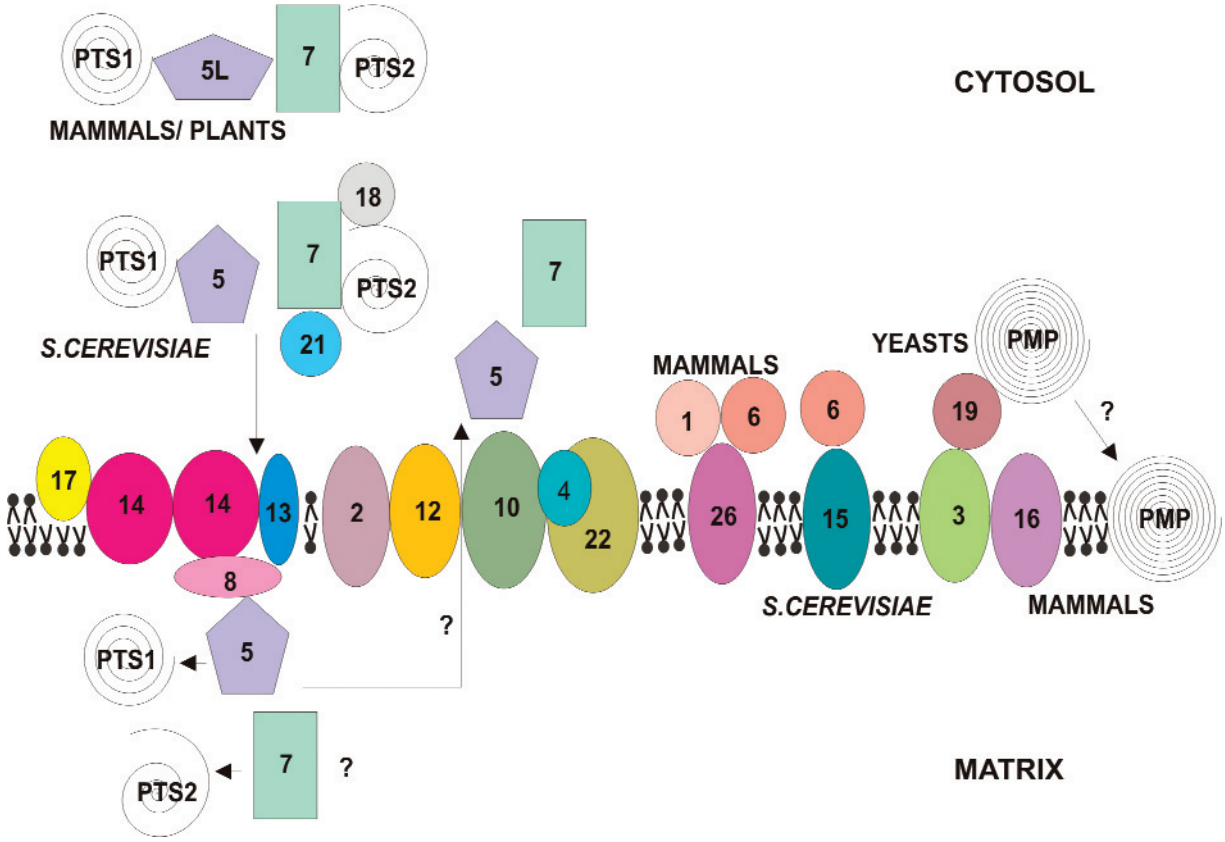

Fig. 1 Overview of protein import into peroxisomes in yeasts, plants and mammals. Matrix proteins have either a PTS1 or PTS2 targeting signal that binds to a cytosolic receptor (with or without accessory proteins, depending on the receptor and the organism) and targets the protein to the docking and translocation machinery localised in the peroxisome membrane. Translocation of the protein may or may not result in the translocation of the PTS2 receptor (Pex7p), although the PTS1 receptor (Pex5p) is inserted into the membrane to such an extent that it binds with the protein, Pex8p, on the *trans* side of the peroxisomal membrane. The receptors are recycled back to the cytosol, possibly via the RING finger complex, which together with the docking and translocation complex is thought to constitute the matrix protein import machinery which strictly requires the presence of Pex8p. It is also thought Pex22p and Pex4p are involved in Pex5p recycling. Pex1p and Pex6p (ATPases) act upstream of Pex4p and Pex22p, but downstream of receptor docking and translocation, thus may also be involved in receptor recycling, although they have been implicated in a number of other roles. It is thought that they are recruited to the peroxisome membrane by Pex15p in *S. cerevisiae* [95] and Pex26p in mammals [96]. Membrane proteins are imported into the peroxisomal membrane by a pathway different to that of matrix proteins. It is possible that Pex3p acts as the receptor for Pex19p, which in turn is thought to be either a chaperone or cycling receptor for PMPs. Together with Pex16p (in mammals) it is proposed that they facilitate the insertion of PMPs into the peroxisomal membrane. For other numbers, see text.

geting signals is PTS1, a carboxy-terminal tripeptide motif which generally complies to the consensus sequence (S/A/C)(K/R/H)(L/M) [11]. The predominately cytosolic protein, Pex5p, is the receptor for PTS1 and structurally, it can be divided into two main domains. In the carboxy-terminal half there is a high affinity PTS1-binding site that contains seven tetratricopeptide repeats (TPRs) [12]. The crystal structure of human Pex5p co-crystallised with a PTS1-containing peptide reveals two clusters of three TPRs (TPR1-3 and TPR5-7) almost completely enclosing the peptide [12]. TPR4, on-the-other-hand, forms a hinge region and is not thought to be directly involved in PTS1 binding [13]. The amino-terminal half of Pex5p has only a few strictly conserved residues, typically in the

multiple pentapeptide WXXXF/Y repeats [14]. It is in this half that the peroxisomal targeting information is contained and is involved in an intricate network of protein-protein interactions [14-16].

The second peroxisomal targeting signal (PTS2) is a nonapeptide and is located near the amino-terminus (usually within 20 residues from the beginning of the chain). It consists of the very general consensus sequence (R/K)(L/V/I)X_5(H/Q)(L/A) and is only present in a small number of matrix proteins such 3-Ketoacyl-CoA thiolase [17]. The receptor for PTS2 is the cycling protein Pex7p, which is characterised by the presence of six WD40-motifs [18]. Pex7p was first discovered in the yeast *Saccharomyces cerevisiae* [19] and has now been described in humans, various fungi and *Arabidopsis thaliana* [20-22]. Interestingly the PTS2 pathway has been completely lost in the nematode *Caenorhabditis elegans* [23]. Although the binding of Pex5p to the PTS1 is well characterised and known to be direct, it is thought that accessory proteins are required in conjunction with Pex7p to for delivery of PTS2-containing proteins to the peroxisome [24] (Fig. 1). In yeast, the structurally related peroxins, Pex18p and Pex21p, are crucial for the import of proteins with PTS2 sequences, although they are partially redundant in function [25]. There are no orthologues of Pex18p and Pex21p in mammals, but studies on Pex5p in Chinese hamster ovary (CHO) cells and human fibroblasts have shown that two splice variants of the protein exists, a short form (Pex5pS) and a long form (Pex5pL) [26]. These two proteins differ by a 37-residue insertion in Pex5pL and although both forms bind PTS1, only Pex5pL interacts with Pex7p. Furthermore, in mammalian cells, protein targeting to the peroxisome requires the not only binding of PTS2 and Pex7p, but also Pex5pL: the disruption of the Pex5pL-Pex7p interaction completely abolishes PTS2 directed import [27]. In addition, the yeast *Yarrowia lipolytica* requires Pex20p for correct PTS2 import [28]. Thus, although there is only weak sequence similarity between Pex5pL, Pex18p/Pex21p and Pex20p, it appears that they may serve a similar function. This is supported by the partial complementation of a *pex18*/*pex21* loss-of-function mutant with Pex20p [28]. A Pex7p homologue has not yet been found in *Y. lipolytic*a, but in *Neurospora crassa* (a filamentous fungus) both Pex7p and Pex20p function together in PTS2-dependent protein import into peroxisomes [29]. The *Arabidopsis PEX5* gene encodes a protein similar to Pex5pL and this interacts with Pex7p [20] suggesting that the PTS1 and PTS2 import pathways may be coupled in plants as they are in mammals.

Although it was previously stated that import into the peroxisome requires either a PTS1 or PTS2, there are deviations. Acyl CoA oxidase (AOX) from *S. cerevisiae* lacks any obvious PTS1 or PTS2 signal and is capable of successful import into the peroxisome. Although no specific signals have been found which can direct AOX to the peroxisome, the interaction between AOX and Pex5p involving novel contact sites in both proteins has been discovered. The interaction region in Pex5p is within the amino-terminal part of the protein, not the TPR domain involved in PTS1 recognition; whilst the interaction site in AOX is located internally, away from the carboxyl-terminus where a PTS1 can normally be found [16].

Docking to the translocation machinery

The next stage in the matrix-protein import journey is the docking of the bound PTS and receptor to the translocation machinery on the peroxisomal membrane. The docking complex includes three peroxins, Pex13p, Pex14p and Pex17p [30] (Fig. 1). Pex17p is a peripheral peroxisomal membrane protein (PMP) which is believed to form part of the docking complex by associating with Pex14p in a particularly tight core complex [30, 31]. Its exact role is not yet known although it was observed in *Pichia pastoris* that *pex14* loss-of-function mutants displayed significantly reduced levels of Pex17p expression and even resulted in Pex17p degradation, supporting the idea that Pex14p and Pex17p interact with each other [32]. Pex13p is an integral PMP that has both its amino and carboxy -termini extending into the cytosol. In *S. cerevisiae* the amino-terminal domain binds the PTS2 receptor (Pex7p) and the carboxy-terminal region contains a Src-homology-3 (SH3) domain which directly binds the PTS1 receptor (Pex5p) as well as the other docking protein, Pex14p [33]. Pex14p is an intrinsic peroxisomal membrane protein (although in *S. cerevisiae* it is reported to be a peripheral peroxisomal membrane protein [34]) thought to be

involved in the docking of Pex5p. Two-hybrid analyses in mammalian cells and complementary *in vitro* binding assays, have shown that the pentapeptide repeat motifs, (WXXXF/Y) in Pex5p bind to Pex14p with high affinity [14, 15].

A second group of membrane proteins, Pex2p, Pex10p and Pex12p, expose their RING-finger domains to the *cis*-side of the peroxisomal membrane and are thought to form a heteromeric complex. Through scrutinising epistatic relationships among several groups of *Pichia pex* mutants, all three proteins were shown to act downstream of receptor docking [35]. The RING-finger peroxins form pairwise interactions and two of them (Pex12p and Pex10p) have been shown to bind to Pex5p [36-38]. The deletion of Pex12p and Pex10p does not result in a decrease in the quantity of Pex5p associated with the peroxisome, implying that Pex12p and Pex10p play no functional role in receptor docking [38].

Through biochemical isolation techniques, the purification and characterisation of two subcomplexes of the peroxisomal import machinery was reported in *S. cerevisiae* [30]. These were found to be (i) the docking complex containing Pex14p, Pex17p and a portion of Pex13p and (ii) the RING-finger complex comprising Pex2p, Pex10p and Pex12p. However, the most intriguing find was that association of both complexes into a larger import complex (the Importomer) strictly required Pex8p, an intraperoxisomal protein previously thought to be involved with the transport machinery, but whose main function was unknown (Fig. 1). The actual function of the RING-finger peroxins has not yet been determined, although it has been proposed that they form a distinct region within the matrix-protein translocation machinery [30, 39]. It has been put forward that docking and translocation do not occur via two separate complexes but through a single dynamic complex. It is also conceivable that the RING-finger complex is involved in receptor re-export (Fig. 1).

In a parallel study using *P. pastoris*, the existence of a complex containing Pex14p, Pex13p, Pex17p and Pex2p, Pex10p and Pex12p was demonstrated [40]. Pex3p was identified as a shared component of both of these subcomplexes (Pex8p was not tested in this study). However, Pex3p is known to be required for the biogenesis of the peroxisome membrane (see later sections) and was shown in this study to be essential for the stability of the RING-finger complex. Hence, the detection of Pex3p as a common component of these two subcomplexes could reflect a role in their assembly rather than directly their function.

Translocation and receptor recycling

Peroxisomes, unlike most other organelles, can import folded and oligomeric protein complexes. Until recently, there was much debate in the peroxisome field over the 'extended-shuttle' and 'simple-shuttle' mechanisms of matrix-protein import [41-43]. The extended shuttle model proposes that the receptor-cargo complex (e.g Pex5p and PTS1 containing protein) translocates freely into the lumen, dissociates in the matrix and the receptor is then exported back into the cytosol for the process to be repeated. The 'simple' shuttle model is similar except that the receptor-cargo complex stays at the surface of the peroxisome (it is not translocated as a complex) and dissociates upon docking. However, due to growing evidence strongly in favour of the 'extended-shuttle' model, the debate now seems to be coming to an end [44]. Pex5p has been shown in mammals to traverse the peroxisome membrane (which is a cargo protein dependant step) and participate in multiple rounds of entry into the peroxisome matrix and export back to the cytosol [45, 46]. Further evidence is provided by the discovery that ScPex5p binds Pex8p: the only known peroxin localised to the *trans* side of the membrane [47]. This shows that Pex5p (with its cargo) can traverse the membrane or at least be inserted into it at such an extent that it is exposed to the *trans* side (Fig. 1).

Other peroxins involved in matrix-protein import

To maintain the process of matrix-protein import, additional peroxins are required to (i) unload the cargo and (ii) return the receptor to the cytoplasm. These peroxins include Pex1p, Pex6p, Pex4p and Pex22p. Pex22p is an integral membrane protein (found only in yeast to date), which anchors the E2-type ubiquitin-conjugating (UBC) enzyme, Pex4p, to the cytosolic face of the peroxisome [48]. In *Hansenula polymorpha*, defects in PTS1 protein

import in mutant cells due to a deficiency in Pex4p can be suppressed by overproduction of Pex5p in a dose-response related manner. This over expression, leads to a build-up of Pex5p in both the cytosol and the inner-face of the peroxisomal membrane and thus suggests a possible role for Pex22p/Pex4p in the recycling of Pex5p [49].

In some ubiquitin E3 ligases, the RING-finger motifs are responsible for bringing the UBC enzymes and their substrates into close proximity. Although at present little is known about the role of the RING-finger- containing peroxins, Eckert & Johnsson [50] have shown that in *S. cerevisia*e Pex10p connects Pex4p to the other members of the protein import machinery. Pex22p is also thought to be involved in this process through maintaining the stability of the Pex10p-Pex4p interaction. Although there are no targets for Pex4p ubiquitination to date, a possible candidate is ScPex18p as it has been shown to be ubiquitinated [25] thus raising the possibility that Pex4p induced ubiquitination is involved in receptor recycling.

Pex1p and Pex6p are two membrane associated ATPases of the AAA (ATPase Associated with diverse cellular Activities) family and are the only known ATPases required for matrix-protein import. According to Collins *et al.* [35] they function upstream of Pex4p and Pex22p, but downstream of receptor docking and translocation. This is consistent with findings by Oliveira *et al.* [51] which suggest that the insertion of Pex5p into the peroxisomal membrane does not require ATP hydrolysis and hence ATP is needed in processes such unloading the cargo and returning the receptor to the cytoplasm (Fig. 1).

Preimplex theory

As previously mentioned, peroxisomes are unusual in that they can import folded proteins and oligomeric protein complexes. They also appear to lack intraperoxisomal chaperones that could either promote the folding of unfolded import substrates or help pull proteins across membranes. Current models have been useful so far for assigning general functions to known peroxins, but lack mechanistic details such as the role of ATP in matrix protein import. A recent hypothesis, the 'preimplex model', aims to address some of these issues and provide testable hypotheses [44]. The preimplex model is based on the idea that peroxisomal matrix-proteins could form large complexes with oligomeric Pex5p (either directly or indirectly depending on the PTS) shortly after their synthesis but before their import. These pre-import complexes of peroxisomal matrix-protein are thus termed 'preimplexes'. Gould and Collins, see preimplex formation as "a stochastic process that is controlled primarily by the concentration of the relevant proteins in the cytoplasm and their affinity for one another". Interestingly, soluble and peroxisome membrane associated complexes of peroxisome matrix proteins were seen in the yeast *Candida boidinii* under conditions of ATP depletion [52]. As mature peroxisomal enzymes are not in large complexes, disassembly must occur either before or during translocation. Pex1p and Pex6p could be involved in this process [44]. The role of Pex1p and Pex6p within the preimplex model, as suggested by Gould and Collins, is to facilitate not only just preimplex disassembly but also the assembly of translocation apparatus and the vectorial transport of proteins across the membrane. However, it should be noted that ATP depletion is likely to have many deleterious effects not least inhibition of cytosolic chaperone function. Fig. 2 shows how the 'extended-shuttle' model would operate within the preimplex hypothesis, contrasting it with the 'trap-door' model (essentially the same as the 'extended-shuttle', only Pex5p is not released into the lumen).

Protein import into peroxisomes: membrane proteins

Targeting of PMPs to the peroxisome

In comparison to matrix protein import, relatively little is known of the targeting and import of peroxisomal membrane proteins (PMPs). Like matrix proteins, many PMPs are synthesised on free polyribosomes and imported post-translationally from the cytosol. However, PMP import does not appear to require the hydrolysis of ATP [53, 54] Mutations in genes that abolish matrix protein import do not usually prevent the insertion of membrane proteins into peroxisome remnants or 'ghosts' and a translocation intermediate that

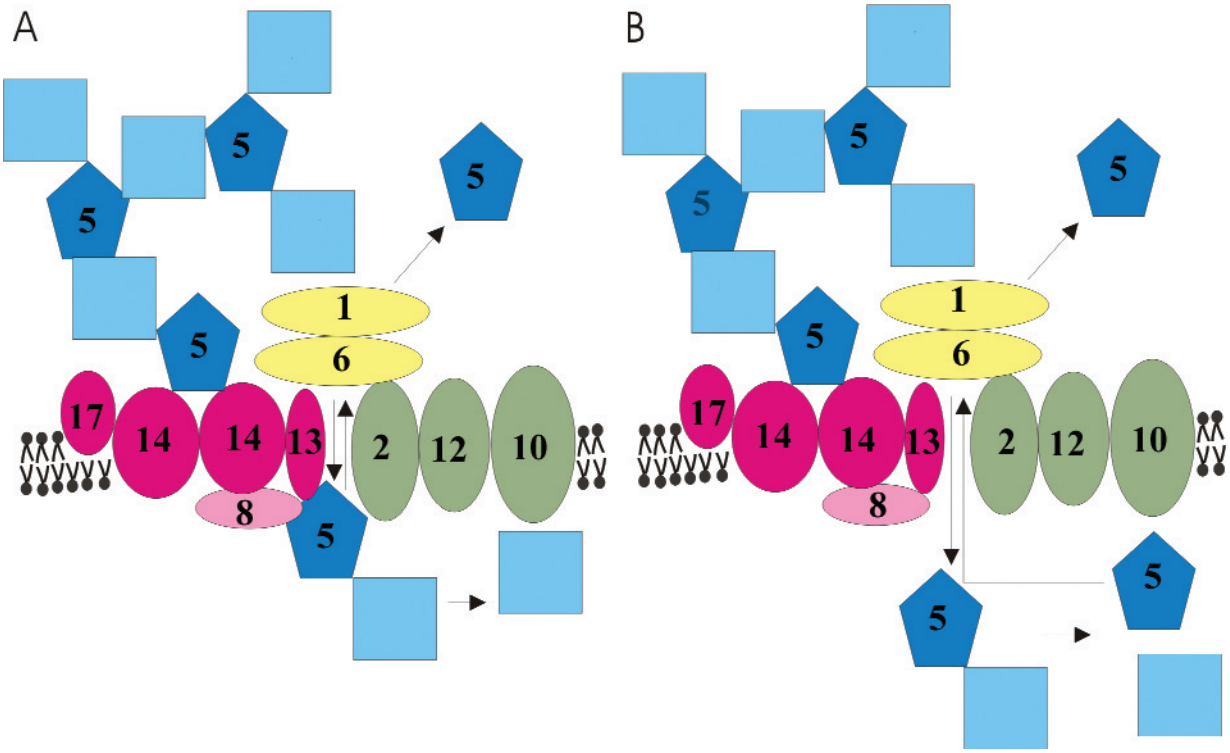

Fig. 2 The 'trap-door' model (A) and the 'extended-shuttle' model (B) operating within the preimplex hypothesis (Adapted from [44]). The PTS1 receptor (Pex5p, dark blue) is thought to behave as an oligomer with several PTS1 binding sites. This results in the formation of a large receptor:protein complex (termed the preimplex) at the surface of the peroxisome membrane. It has been propsed the AAA ATPases Pex1p and Pex6p (yellow) facilitate preimplex disassembly before translocation. In the 'trap-door' model Pex5p only enters the peroxisome as far as to bind to the protein, Pex8p (on the *trans* side of the peroxisomal membrane), whilst in the 'extended-shuttle' model the receptor is thought to enter the actual peroxisomal matrix before being recycled back into the cytosol.

blocked binding of matrix proteins to isolated glyoxysomes did not affect binding of membrane proteins [55]. These observations suggest that PMPs are imported by a mechanism distinct from that employed by matrix proteins. Several studies have attempted to define the targeting information in PMPs, although only in a few cases has a small defined sequence been identified as a membrane targeting signal (mPTS). These mPTSs have been described for Pex3p from mammals and yeast, CbPmp47p, HsPmp34p, cottonseed ascorbate peroxidase (APX) and Pmp22p from mammals and plants. It should be noted though that these sequences do not exhibit a clear consensus.

Pex3p contains a putative transmembrane domain (TMD) in its amino-terminal region and is thought to be firmly anchored in the peroxisomal membrane with the remainder of the protein exposed to the cytosol. Studies in several species (*H. polymorpha*, *Pichia* and human) have shown that the targeting information of Pex3p is found within the amino-terminal region [56]. Here there is a conserved block of 3-4 basic residues which are similar to the centre of the Pmp47p loop (next paragraph). Through the comparison of mammalian and yeast forms of Pex3p, Baerends *et al.* (2000) suggested the consensus sequence of RX(K/R)XK [57].

In *C. boidinii*, Pmp47p consists of six TMDs (two loops on the matrix side and three on the cytoplasmic side of the membrane). Dyer *et al.* (1996) reported that the Pmp47p targeting information resides within the second matrix loop, which contains a basic cluster of amino acids of the sequence KIKKR [58]. More recently, similar sequences in other peroxisomal membrane proteins have been

recognised (such as Pmp70p and Pmp3p) and a consensus sequence of XX(K/R)(K/R)3-7X(T/S) XX(D/E)X has been proposed [59]. In addition, further examination into the targeting signals of CbPmp47p has also revealed that TMD2 plus an adjacent cytoplasmic-orientated sequence is crucial for efficient targeting [59].

Human Pmp34p is a putative orthologue of *C. boidinii* Pmp47p but interestingly, has the opposite topology with the amino and carboxyl termini in the cytosol. Honsho & Fujiki, (2001) suggested that the positively charged loop between TMD4 and 5 (corresponding to the region that functions as a mPTS in CbPMP47) is a potential mPTS [60]. However, this alone is insufficient for targeting and the loop together with three TMDs is needed for targeting and insertion into the peroxisome membranes. On the other hand, Jones *et al,*. (2001) demonstrated that Pmp34p contains at least two nonoverlapping sets of targeting signals, either of which is sufficient for insertion into the peroxisome membrane [61]. They also showed that two non-overlapping segments of PEX13 target to peroxisomes, thus indicating that the presence of multiple independent targeting regions within a single PMP may not be unique to Pmp34p.

Peroxisomal APX is a carboxy-terminal tail-anchored integral PMP. Transient expression of mutant forms of APX showed that the carboxy-terminal 5 amino acid residues (RKRMK-COOH) were minimally necessary for localisation to the peroxisome membrane. The substitution of the peroxisomal APX TMD with an artificial TMD (lacking any sorting sequences) plus the peroxisomal APX carboxy-terminal tail sorted chloramphenicol acetyltransferase to peroxisomes. This suggests that the peroxisomal APX TMD does not possess essential sorting information but instead confers the context required for the conserved positively charged domain to function within peroxisomal APX [62].

Pmp22p is a 22-kD peroxisomal membrane protein family of unknown function, although studies with mouse family members Mpv17 [63] and M-LP [64] suggests that they are involved in enzymic antioxidant defence systems. Pmp22p contains four putative TMDs and both termini are exposed to the cytosol. Pause *et al.* [65] suggested that the amino-terminal 37 amino acids in rat Pmp22p is a possible mPTS, among which the first 15 are important for efficiency but only function in the context of more than one TMD. Comparison with orthologous proteins revealed the conserved motif Y3xL3xP3x(KQN), which was suggested to represent the core of the signal sequence. Recently it was demonstrated that at least four distinct regions within the amino-terminal half of *Arabidopsis* Pmp22p (including a positively charged domain, KRKK, similar to that present in most PMPs and the Y3xL3xP3xK motif that is shared with mammalian Pmp22p) function in a co-operative manner in correct insertion and assembly in the peroxisomal membrane [66]. In addition, targeting with high fidelity to peroxisomes also requires all four TMDs.

Therefore, in PMPs that have a single membrane span (such as Pex3p), putative mPTSs appear to have a hydrophilic peptide which contain a group of positively charged amino acids adjacent to at least one hydrophobic patch or TMD, although there is no common amino acid sequence among them. In multi spanning proteins (such as Pmp22p, Pmp47p and Pmp34p), putative mPTSs appear to be more complex. Here the co-operation of multiple regions of the protein is necessary for efficient targeting and at least one TMD and loop or tail region (which can reside on either side of the membrane) appears to be important for localisation and insertion. As insertion entails the anchoring of PMPs into the membrane, it is of no coincidence that the presence of a transmembrane span is a common property of mPTSs.

PMP import machinery

It is expected that mutations in putative peroxins involved in PMP integration into the peroxisomal membrane would result in the depletion of most of the peroxisomal membrane's usual complement of proteins and even the entire loss of the peroxisomal membrane. Pex19p, Pex3p and Pex16p are thought to be essential for the assembly of peroxisome membranes as peroxisomal ghosts are undetectable in mammalian *pex19, pex3* and *pex16* mutants cells, despite otherwise normal synthesis of membrane proteins. However, this is not always the case in other organisms. Ghosts are present in *P. pastoris* loss-of-function *pex3* mutants [40] as well as *Y. lipolytica* and *P. pastoris* loss-of-function *pex19* mutants [67, 68]. Nonetheless, it is still

thought that Pex19p, Pex3p and Pex16p play a critical role in the biogenesis of the peroxisomal membrane (Fig. 1).

Pex19p is predominately cytosolic although it can also be found transiently associated with the peroxisomal membrane, likely via a farnesyl group attached to the carboxy-terminus [68]. It has been shown to interact with the cytosolic portion of Pex3p as well as a broad spectrum of other PMPs that are unrelated to peroxisome biogenesis [69, 70]. Observations regarding (i) the cellular distribution of Pex3p and Pex19p, (ii) the similar loss-of-function phenotypes of *pex3* and *pex19* mutants and (iii) the binding of Pex19p to a number of PMPs has lead to the proposal that Pex19p may function as a import receptor for newly synthesised PMPs [70, 71]. Here, Pex19p recognises and binds to PMPs in the cytosol and targets the PMPs to the peroxisomal membrane with Pex3p as a possible docking factor (Fig. 1).

Determining whether Pex19p interacts with the targeting sequences of PMPs is a crucial step towards establishing whether Pex19p is indeed a cycling receptor. One detailed study showed that multiple independent point mutations altered HsPex19p binding affinity to HsPex13p by 3-4 orders of magnitude but did not abolish peroxisomal targeting [72]. This suggests that HsPex13p binding to Pex19p is independent from peroxisomal targeting. Furthermore, it has been seen in *P. pastoris* that Pex19p generally interacts with existing PMPs (opposed to newly synthesised PMPs) and that these interactions occur predominantly at the peroxisome membrane [70]. This suggests that rather than acting as a soluble receptor, Pex19p may perform a chaperone-like role at the peroxisome membrane, although this is still somewhat of a contentious view.

Pex16p has also been implicated in the assembly of peroxisomal membranes. Human cells that are deficient in Pex16p do not have detectable peroxisome ghosts. Although the role of Pex16p in PMP targeting is still unknown, Honsho *et al.,* (2002) recently suggested that Pex16p may function at an early stage of peroxisome membrane assembly upstream of Pex3p and Pex19p [73]. Possible roles included a component of the membrane translocator, or alternatively, a receptor for PMPs including Pex16p itself, or more simply, exclusively Pex3p.

Biogenesis and proliferation

Biogenesis

The classic model of peroxisome biogenesis entails peroxisomes solely arising through the fission of pre-existing peroxisomes [10]. In recent years this view has been challenged by a number of groups who believe that in addition to the fission of 'late and mature' peroxisomes there is also *de novo* synthesis of 'early and immature' peroxisomes (already reviewed in [39, 56, 74, 75]). Currently, it is thought that Pex3p in these 'early' peroxisomes acts as the receptor for Pex19p (thought to be either a cyclic receptor or chaperone for PMPs) that (along with Pex16p in humans) subsequently allows the insertion of PMPs, giving rise to fully developed and functional 'late' peroxisomes (Fig. 1). The actual source of such 'early' peroxisomes is still under debate, with the ER suggested as a possible membrane source (reviewed in depth by [56, 74, 76]).

Proliferation

Peroxisomes are dynamic organelles, undergoing proliferation, movement and turnover in response to extracellular (and probably also intracellular) stimuli [76]. There appear to be three mechanisms that impact on peroxisome abundance and size. Precise details are still lacking, as is our understanding of how these mechanisms are related to one another and whether they are universal. These mechanisms are: (1) Promotion of division by a mechanism involving the peroxisomal membrane protein Pex11p [77-83] and dynamin-like proteins [84, 85]; (2) Inhibition of division by Pex16p in *Y. lipolytica*, which is relieved by binding of a heteropentameric complex of acyl-CoA oxidase to Pex16p [86]. This binding is stimulated by the matrix protein content of the peroxisome and is an attractive mechanism by which peroxisomes can measure their internal volume and divide at an appropriate size; (3) Metabolic control over peroxisome size or abundance, in which various β-oxidation mutants have reduced numbers of enlarged peroxisomes [87-91].

Interestingly, in mice with loss-of-function Pex11a and Pex11b, numerous peroxisomes still

exist and no obvious peroxisomal protein import defects are exhibited [82]. However, these knock-out mice die early after birth due to severe neurological defects, thus suggesting that Pex11p is involved in another role other than peroxisome proliferation.

Two novel proteins, Pex25p and Pex27p, have also been associated with the control of peroxisome size and number in *S. cerevisiae* [92-94]. The C-termini of these homologous proteins are similar to the entire Pex11p. Deletion of either protein results in enlarged peroxisomes and overexpression results in peroxisome proliferation and formation of small peroxisomes [93, 94]. Interestingly, a severe peroxisomal protein import defect was observed in triple *pex11*, *pex25* and *pex27* loss-of-function mutants even when cells were grown on ethanol-containing medium. As peroxisomes are not required when cells are grown on ethanol-containing medium, it is thought that these proteins play a role in peroxisome biogenesis other than the control of peroxisome proliferation and size [93].

Conclusion

Although much progress has been made in recent years regarding the isolation and characterisation of the proteins catalysing the biogenesis of peroxisomes, functional data is still lacking with many studies reporting conflicting results. Hopefully over the next few years progress in molecular and biophysical approaches will allow a clearer picture of peroxisomal protein import to emerge, allowing many important questions to be addressed (such as the nature of the translocation machinery and the role of specific components like Pex1p and Pex6p) and also to see the advancement of possible therapies in treating peroxisomal biogenesis disorders.

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