Review

Targeting signals in peroxisomal membrane proteins

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Abstract

Peroxisomal membrane proteins (PMPs) are encoded by the nuclear genome and translated on cytoplasmic ribosomes. Newly synthesized PMPs can be targeted directly from the cytoplasm to peroxisomes or travel to peroxisomes via the endoplasmic reticulum (ER). The mechanisms responsible for the targeting of these proteins to the peroxisomal membrane are still rather poorly understood. However, it is clear that the trafficking of PMPs to peroxisomes depends on the presence of cis-acting targeting signals, called mPTs. These mPTs show great variability both in the identity and number of requisite residues. An emerging view is that mPTs consist of at least two functionally distinct domains: a targeting element, which directs the newly synthesized PMP from the cytoplasm to its target membrane, and a membrane-anchoring sequence, which is required for the permanent insertion of the protein into the peroxisomal membrane. In this review, we summarize our knowledge of the mPTs currently identified.

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1. Introduction

Proteins destined for import into the peroxisomal matrix or insertion into the peroxisomal membrane follow distinct pathways [1]. This review deals with the targeting of peroxisomal membrane proteins (PMPs) from the cytoplasm, where they are synthesized, to their final destination, the peroxisomal membrane. Currently, two targeting routes for PMPs have been identified: some are targeted from the cytoplasm directly to the peroxisomal membrane (cytoplasm-to-peroxisome pathway) [2]; others are sorted indirectly to peroxisomes by way of ER-derived vesicles or a specialized subdomain of the ER, the peroxisomal endoplasmic reticulum (pER) (pER-to-peroxisome pathway) [3,4]. During and after synthesis, PMPs must avoid aggregation and maintain their import-competent conformation. The emergent view is that PMPs are bound by soluble factors, shuttled to the target membrane, handed over to a membrane insertion apparatus, and inserted into the membrane. How these events occur and what molecules and mechanisms are involved are beginning to be understood. However, many questions remain. For example, it is not clear whether or not these processes require a source of energy [5–8]. What is clear is that (i) the trafficking of PMPs to peroxisomes depends on the presence of cis-acting targeting signals, and (ii) PMP insertion occurs at proteinaceous sites on the peroxisomal membrane [5,7]. As the proper biogenesis of a membrane protein requires targeting and membrane insertion, the term ‘mPTS’ will hereafter refer to a region of a PMP that has the capability to both target the protein to, and insert it into the peroxisomal membrane [9].

2. Overview of the currently identified mPTs

2.1. Amino-terminally anchored proteins

2.1.1. Pex3p

Pex3p is a PMP that functions in peroxisome membrane assembly. The number of predicted transmembrane domains as well as the topology of the protein varies between species [10,11]. Despite these differences, the targeting information of all Pex3p orthologues seems to be contained within their amino-terminal 46 amino acids [10–15]. An alignment of these protein
sequences suggests that the core architecture of the Pex3p mPTS consists of a hydrophobic domain immediately preceded by a cluster of basic amino acid residues (Table 1). The observations that the hydrophobic domain and the cluster of positively charged amino acids of Homo sapiens (Hs) and Arabidopsis thaliana (At) Pex3p are sufficient for directing a heterologous reporter protein to peroxisomes in both human skin fibroblasts and Arabidopsis suspension-cultured cells, respectively, are in excellent agreement with these predictions [10,11]. Importantly, neither the hydrophobic domain nor the stretch of positively charged amino acids alone is sufficient for peroxisomal localization [10,11]. Also, as single or double amino acid substitutions within the stretch of basic amino acids do not interfere with Pex3p localization, none of the positively charged amino acids seem to be essential for targeting [11,14].

2.1.2. Pex14p

Pex14p functions as the initial docking site for the import receptors of peroxisomal matrix proteins at the peroxisomal membrane. The protein contains one hydrophilic segment in its amino-terminal domain, is attached to the outer surface of the peroxisomal membrane in Saccharomyces cerevisiae (Sc) [16], and behaves as an intrinsic membrane protein in mammals [17]. Currently, only the mPTS of human Pex14p has been delineated (Table 1). The topogenic information of this protein is situated within the N-terminal 25 amino acids (1 transmembrane segment, N→C) PMP that is involved in the recruitment of the Pex6p AAA ATPase to the peroxisomal membrane [26]. Pex15p has been identified only in baker’s yeast, and overexpression of the protein causes a profound proliferation of the ER membrane [27]. Deletion analysis studies have shown that the signal directing ScPex15p to peroxisomes is located between amino acids 302 and 371 [27].

2.1.3. Pex17p

Pex17p is a peroxisomal membrane protein that acts primarily in the matrix protein import pathway. It contains one putative transmembrane segment near the amino-terminus, behaves as a peripheral membrane protein that is tightly bound to the outer surface of the peroxisomal membrane in S. cerevisiae [22], and as an integral membrane protein in Pichia pastoris (Pp) [23]. While the mPTS of ScPex17p is not yet mapped, it has been demonstrated that the targeting information of PpPex17p is contained within the N-terminal 56 amino acids of the protein [24]. This region of Pex17p contains a hydrophilic domain which, in contrast to the hydrophobic domain within the Pex3p mPTS, is not immediately preceded by a cluster of positively charged amino acids (Table 1). Whether or not the hydrophobic domain and the randomly distributed positively charged amino acids are essential for targeting, remains to be investigated.

2.1.4. Pex22p

Pex22p is an amino-terminally anchored type I (1 transmembrane segment, N→C) PMP that anchors the ubiquitin-conjugating enzyme, Pex4p, to the peroxisomal membrane. Currently, the protein has only been identified in the yeasts P. pastoris and S. cerevisiae [25]. The mPTS of PpPex22p resides within the N-terminal 25 amino acids of the protein. This region is characterized by the presence of a putative transmembrane segment that is immediately preceded by a stretch of positively charged amino acids (Table 1). The observation that PpPex22p(1–25)−GFP fusions with alanine substitutions in two of the three positively charged amino acids do not properly localize to the peroxisome, suggests that at least two positive charges are important for proper targeting [25]. And, as is the case for Pex3p, the cluster of positively charged amino acids or the hydrophobic domain alone is not sufficient for peroxisomal targeting [25].

2.2. Tail-anchored proteins

2.2.1. Pex15p

Pex15p is a tail-anchored, type II (1 transmembrane segment, N→C) PMP that is involved in the recruitment of the Pex6p AAA ATPase to the peroxisomal membrane [26]. Pex15p has been identified only in baker’s yeast, and overexpression of the protein causes a profound proliferation of the ER membrane [27]. Deletion analysis studies have shown that the signal directing ScPex15p to peroxisomes is located between amino acids 302 and 371 [27].
The same authors have shown that (i) the amino acids 354 to 371 of ScPex15p are required, but not sufficient, for peroxisomal targeting, and (ii) the amino acids 302 to 353 contain ER targeting information. Note that the region of ScPex15p flanked by the amino acids 302 and 371 contains a putative transmembrane segment, which is immediately followed by a matrix-oriented cluster of basic amino acids (Table 2). Whether or not this cluster of basic amino acids, the putative transmembrane segment or the two clusters of basic amino acids present in the cytoplasmically oriented part of the targeting domain are essential for peroxisomal localization, remains to be determined.

2.2.2. APX

Peroxisomal ascorbate peroxidase (APX) is a carboxyl tail-anchored, type II PMP that functions in the regeneration of NADPH in glyoxysomes of germinated oilseeds and the protection of peroxisomes from toxic hydrogen peroxide in other organisms [28]. It has been shown that Gossypium hirsutum (Gh) APX (i) is inserted posttranslationally into ER membranes – and not into peroxisomal membranes – in vitro [6], and (ii) sorts indirectly via a subdomain of the ER to the boundary of peroxisomes in Bright Yellow-2 tobacco cells [29]. The sorting information of GhAPX is contained within its C-terminal 26 amino acids [28]. This region of GhAPX, which consists of a predicted transmembrane segment immediately followed by a cluster of positively charged amino acids (Table 2), is sufficient to target a heterologous reporter protein from the cytosol to peroxisomes via the pER. Note that the stretch of basic amino acids is essential, but not sufficient for targeting. Also, there is no strict requirement for a single basic amino acid residue or a specific sequence of residues within the positively charged domain [28]. Interestingly, the predicted transmembrane segment can be replaced with an artificial hydrophobic domain devoid of putative sorting sequences without losing targeting function. Therefore, the putative transmembrane domain of GhAPX does not possess essential sorting information but rather serves to present the positively charged amino acids in their proper context [28].

2.2.3. Fis1p

HsFis1p is a carboxy-terminal tail-anchored type II integral membrane protein involved in mitochondrial and peroxisomal fission [30,31]. The C-terminal 26 amino acids, which comprise the putative transmembrane segment and a short five- amino acid tail (Table 2), contain both peroxisomal and mitochondrial targeting information [31]. Substitution of the two lysines in the C-terminal tail with alanines, results in a disturbed mitochondrial targeting [30]. Whether or not these lysine residues are also essential for peroxisomal targeting, is unknown.

2.3. Multispanning membrane proteins

2.3.1. RING finger peroxins

Pex2p, Pex10p, and Pex12p are integral membrane proteins containing a C3HC4 RING finger motif in the C-terminus. For HsPex2p, it has been shown that the region between amino acids 114 and 185 is necessary and sufficient for peroxisomal targeting [32]. This region is characterized by the presence of a cluster of positively charged amino acids and the first putative transmembrane segment (Table 3). Addition of a second (artificial) hydrophobic domain increases the targeting efficiency of this fragment, and under these conditions, the cluster of basic amino acids is dispensable for targeting [32]. On the other hand, all HsPex2p deletion constructs displaying a peroxisomal localization contain amino acids 130 to 159, suggesting that these amino acids form the core of the HsPex2p mPTS [32]. Depending on the organism, Pex2p is sorted to the ER en route to the peroxisomes [33], or targeted to peroxisomes independently of known ER trafficking routes [2]. AtPex10p has also been shown to be targeted to peroxisomes independently of known ER trafficking routes [2]. Currently, only the mPTS of PpPex10p has been delineated [24]. This mPTS is characterized by the presence of the putative second transmembrane segment preceded by a cluster of positively charged amino acids (Table 3). For HsPex12p, it has been reported that the sorting information is contained between amino acid residues 50 and 233 [34]. Note that the RING finger domain is not necessary for targeting Pex2p, Pex10p, and Pex12p to peroxisomes.

2.3.2. The Pex11p family

Members of the Pex11p family are known to regulate peroxisome size and number in multiple species [35]. Currently, the mPTSs of three members of this family – HsPex11pβ, ScPex30p, and ScPex32p – have been partially delineated. The targeting information of HsPex11pβ is situated between amino acids 181 and 259 (Table 3) [18]. The targeting

<table>
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<th>PMP</th>
<th>Amino acid sequence</th>
<th>Pex 19p</th>
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<tr>
<td>ScPex15p</td>
<td>302 TGTAPRKKXNDITVLAGSFWAVLKHFTRVWKNQGLLTCGLLLCGLKLYKSLMAIFKH VPAAFHTVYP</td>
<td>+</td>
</tr>
<tr>
<td>GhAPX</td>
<td>263 OA VGV AAAAAVIVLSY FE VRKM</td>
<td>?</td>
</tr>
<tr>
<td>HsFis1p</td>
<td>127 AIVVGMALVGAVGLAGLCLAVKSSKS</td>
<td>?</td>
</tr>
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</table>

For each mPTS, the residue number of the first amino acid is listed. Predicted transmembrane segments (by TMPRED, scores above 500 [65]) are boxed, predicted Pex19p-binding sites (by a yeast-based prediction matrix [38]) are indicated in red, and clusters of positively charged amino acid residues (window size 6; match > 3/6) are shaded in blue. Abbreviations: Gh, Gossypium hirsutum; Hs, Homo sapiens; PMP, peroxisomal membrane protein; Sc, Saccharomyces cerevisiae; +, interaction; ?, unknown.
information of ScPex30p and ScPex32p has been reported to be situated between amino acids 230–250 and 159–179, respectively [36]. However, although Pex30p(230–250) and Pex32p(159–179) are sufficient to target a reporter protein to peroxisomes, the targeting efficiency is very low [36]. This is not unexpected as these constructs do not contain a hydrophobic segment [36]. Also, the amino acids 230 to 250 of Pex30p are not essential for peroxisomal localization [36], suggesting that this peroxin contains multiple mPTSs.

### 2.3.3. Pex13p

Pex13p is an essential component of the peroxisomal matrix protein import machinery. The protein possesses two putative transmembrane segments and contains an SH3-domain in its cytoplasmically exposed C-terminus [37]. The targeting information of PpPex13p is contained between the amino acids 178 and 251 [24]. For HsPex13p, it has been shown that the central matrix loop (amino acids 155 to 233) contains sufficient information to target a reporter protein to peroxisomes, albeit inefficiently [36]. However, increasing the hydrophobicity of this loop by adding one of the putative flanking transmembrane segments enhances the overall sorting efficiency [34]. Also, the fragment between amino acids 70 and 140 includes a cluster of basic amino acids that is essential, but not sufficient for peroxisomal localization [36], suggesting that this peroxin contains multiple mPTSs.

### 2.3.4. Pex16p

Depending on the organism, Pex16p behaves as an integral [40] or intraperoxisomal peripheral [41] membrane protein. HsPex16p contains two putative transmembrane segments and both the N- and C-terminal parts of the protein are exposed to the cytoplasm [40]. Currently, two fragments of HsPex16p have been reported to contain targeting information: amino acids 70 to 140 [40] and amino acids 221 to 336 [42]. Each fragment contains one putative transmembrane information (Table 3). Also, the fragment between amino acids 70 and 140 includes a cluster of basic amino acids that is essential, but not sufficient for peroxisomal targeting [40].

### 2.3.5. PMP22/M-LP

PMP22 proteins contain four putative transmembrane segments, and for Rattus norvegicus (Rn) PMP22 it has been shown that the amino- and carboxy-termini face the cytoplasm [43]. Although it was first claimed that the targeting information of RnPMP22 is contained between amino acids 16 and 37, sufficiency of this region to target the protein to peroxisomes was never shown [44]. However, fusion of the first 37 amino acids to amino acids 94–194, a fragment which failed to be targeted in the hands of this group, restored its peroxisomal localization. Deletion analysis studies by a different group subsequently demonstrated that HsPMP22 (Table 4) and RnPMP22 (data not shown) have two nonoverlapping mPTSs, both necessary and sufficient for targeting a reporter protein to the peroxisomal membrane [45]. AtPMP22 was reported to...
have only one single mPTS. Although amino acids 1–78 were necessary and sufficient for partial peroxisomal localization, almost the entire protein was necessary for efficient targeting [46].

Mus musculus (Mm) M-LP (Mpv17-like protein), a PMP which may be involved in the metabolism of reactive oxygen species [47], shows a high sequence similarity with the peroxisomal membrane protein HsPMP22 and the inner mitochondrial membrane protein Mpv17 [48]. M-LP has three putative transmembrane segments but its precise topology in the peroxisomal membrane has not been determined. The mPTS of MmM-LP has been mapped between amino acids 16 and 55. This region, consisting of the first predicted transmembrane segment and a cluster of basic amino acids contained in the loop between the first two putative transmembrane segments (Table 4), is both necessary and sufficient for targeting a reporter protein to peroxisomes [47].

2.3.6. Peroxisomal solute carriers

Candida boidinii (Cb) PMP47 and HsPMP34 are peroxisomal homologues of mitochondrial solute carrier proteins. Although both proteins contain six putative transmembrane segments, they display opposite topologies in the peroxisomal membrane [49,50]. Initially, it was claimed that the basic 20 amino acid loop present between the fourth and the fifth predicted transmembrane segments of CbPMP47 is both necessary and sufficient for peroxisomal targeting [51]. However, researchers from the same laboratory later reported that this loop by itself is targeted to peroxisomes only very poorly, and that high fidelity targeting of CbPMP47 to (oleate-induced) peroxisomes requires the concerted action of (i) the second predicted transmembrane segment (amino acids 95 to 110), (ii) the adjacent cytoplasmically oriented sequence (amino acids 70 to 94), (iii) a short matrix loop containing a cluster of basic amino acids, and (iv) a second membrane-anchoring hydrophobic domain [52]. Interestingly, replacement of amino acids 70 to 110 with the fourth predicted transmembrane segment (amino acids 203 to 224) and its adjacent cytoplasmically oriented sequence (amino acids 176 to 202) yields an mPTS sufficient for targeting a reporter protein to basal, but not to proliferated peroxisomes [53]. Currently, there is no consensus on the targeting signal(s) of HsPMP34. On one hand, it has been reported that the loop by itself is targeted to peroxisomes only very poorly, and that high fidelity targeting of CbPMP47 to (oleate-induced) peroxisomes requires the concerted action of (i) the second predicted transmembrane segment (amino acids 95 to 110), (ii) the adjacent cytoplasmically oriented sequence (amino acids 70 to 94), (iii) a short matrix loop containing a cluster of basic amino acids, and (iv) a second membrane-anchoring hydrophobic domain [52]. Interestingly, replacement of amino acids 70 to 110 with the fourth predicted transmembrane segment (amino acids 203 to 224) and its adjacent cytoplasmically oriented sequence (amino acids 176 to 202) yields an mPTS sufficient for targeting a reporter protein to basal, but not to proliferated peroxisomes [53].

### Table 4

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protein depends on the hydrophilic loop between the fourth and fifth predicted transmembrane segment and (at least) three membrane-anchoring segments [50]. On the other hand, it has been demonstrated that HsPMP34 contains two non-overlapping mPTSs (amino acids 1–147 and 255–307) (Table 4), each of which is sufficient for insertion into the peroxisomal membrane [39]. Note that neither mPTS reported by Jones et al. [39] contains the hydrophilic loop which was claimed to be essential for PMP34 targeting by Honsho and Fujiki [50]. One way to explain these apparently conflicting conclusions is that HsPMP34 could contain three distinct, but overlapping targeting signals.

2.3.7. ABC transporters

ATP-binding cassette (ABC) transporters of the peroxisomal membrane (ABC subfamily D) are — with the exception of one plant representative — ‘half-transporters’ consisting of an amino-terminal transmembrane domain with six predicted transmembrane segments fused to a nucleotide binding domain. Several lines of evidence suggest that the peroxisomal ABC transporters are involved in the transport of activated fatty acids/carboxylates across the peroxisomal membrane [54]. In mammals, the ABCD subfamily comprises four members: the adrenoleukodystrophy protein (ALDP), the ALDP-related protein (ALDRP), PMP69, and PMP70 [54]. HsPMP70 bears two distinct fragments (residues 1–61 and 61–138) containing peroxisomal targeting information (Table 4) [18,55]. However, these fragments target a reporter protein rather inefficiently to peroxisomes, and precise targeting exclusively to peroxisomes requires larger fragments (residues 1–124 and 61 to 160) [18,55]. The targeting information of HsPMP69 is contained between the amino acids 16 and 133 (Table 4) [56]. For HsALDP, the amino acids 1 to 110 and 67 to 164 contain sufficient information for targeting a reporter protein to the peroxisomal membrane (Table 4). Interestingly, the overlapping region between these two fragments (amino acids 67 to 110) is essential, but not sufficient for targeting [56]. The mPTS of HsALDRP has not yet been delineated. In S. cerevisiae, the ABCD subfamily comprises two members: Pxa1p and Pxa2p. The targeting information of ScPxa1p, a putative orthologue of HsALDP, is located between the amino acids 71 and 168 [56]. Recently, three glycosomal ABC-half-transporters were identified in Trypanosoma brucei (Tb). The targeting information of two of these transporters, TbGAT1 and TbGAT2, was determined and is contained between amino acids 1–169 and 68–126, respectively (Table 4) [57].

3. Classes of mPTSs

3.1. Multiple sorting pathways

The currently identified mPTSs vary greatly in length, and are unremarkable by primary structure analysis. These observations suggest that the peroxisomal sorting information is not contained within a specific amino acid sequence, but rather within the physicochemical characteristics of the mPTS sequence. Alternatively, this may suggest the existence of distinct PMP import pathways. In this context, it is important to note that some PMPs (e.g. AtPMP22 and HsPMP34) are sorted directly from the cytoplasm to peroxisomes, while other PMPs (e.g. GhAPX and ScPex3p) travel to peroxisomes via the ER (see above). Therefore, the existence of two types of mPTSs was hypothesized. One class might target proteins directly from the cytoplasm to the peroxisomal membrane, and a second class might be involved in directing PMPs from a specialized subdomain of the ER to the peroxisome [37].

3.2. Membrane topology

It can be easily imagined that the import of N-terminally anchored, tail-anchored and multispacing PMPs occurs in a mechanistically distinct manner. However, despite this and the fact that different PMP targeting routes may exist, one common feature of all currently identified mPTSs, is that they are never proteolytically removed upon import. Also, most mPTSs displaying a high targeting efficiency are characterized by the presence of a cluster of basic amino acids and at least one putative transmembrane segment (Tables 1–4). Possible key factors that may determine organelle specificity are the position and number of positively charged amino acids and the length and hydrophobicity of the transmembrane segments. For example, it has been shown that mitochondrial targeting of mammalian tail-anchored proteins generally requires a shorter transmembrane segment (<20 residues) and a tail region enriched in positively charged amino acids, whereas ER targeting requires a longer transmembrane segment with fewer positively charged residues in the tail sequence [58]. Whether or not similar physicochemical parameters are also important for the peroxisomal localization of (tail-anchored) PMPs, remains to be explored. However, for one tail-anchored protein (GhAPX) and a number of multimembrane spanning proteins (HsPex2p, HsPex13p, ScPex13p, CbPMP47, TbGAT2), it has already been shown that the transmembrane segment in the mPTS functions as an anchor (and not as a signal-anchor) sequence [28,32,34,38,53,57]. Currently, nothing is known regarding the factors controlling the final orientation of a transmembrane helix in the peroxisomal membrane. One model that was put forth suggests that the targeting signal in the mPTS essentially acts like a regular PTS in that it is translocated to the matrix side of the peroxisome, while transmembrane segments act as stop-transfer sequences [37]. Specifically, this model implies that it is the relative location of the mPTS within the protein, and not the transmembrane segments, which plays a key role in the overall topology of the protein in the peroxisomal membrane [37]. Alternatively, since in many membrane proteins, the inside/outside location of the polar domains strongly correlates with their lysine and arginine content [59], the positively charged residues present in most mPTSs may determine the topology of the proteins. This view is also supported by the finding that the targeting process itself is not dependent on a single basic amino acid residue or specific sequence of residues within the positively charged domain of an mPTS [11,14,25,28].
3.3. Dependency on Pex19p

Hydrophobic stretches which ultimately form the transmembrane helices and positively charged amino acid residues in the regions flanking them are the two major topological determinants in nearly all prokaryotic and eukaryotic membrane proteins [60]. Therefore, as these features themselves are unlikely to be sufficient to ensure proper PMP localization, one might expect mPTSs to contain more subtle targeting information. In this context, it is interesting to note that the targeting signals of many PMPs interact with Pex19p (see Tables 1–4). Pex19p is essential for the formation of functional peroxisomal membranes, and has been found in the cytoplasm and, to a minor extent, on the surface of the peroxisomal membrane [9]. Recently, it was shown that it is the Pex19p-binding site within the mPTS which contains the targeting information, while the transmembrane segment within the mPTS is required for the permanent insertion of the protein into the peroxisomal membrane [38, 61]. These findings directly imply that Pex19p functions as a cycling receptor protein for newly synthesized PMPs [1]. However, this conclusion needs to be interpreted with care. Indeed, it has been reported that (i) inhibition of Pex19p expression has no effect on the targeting of Pex3p [42], and (ii) the mPTS and Pex19p-binding sites are distinct entities in multiple PMPs [19, 24, 34, 36]. Summarized, these findings suggest that there are at least two classes of functionally distinct mPTSs: class 1 mPTSs, which require Pex19p function, and class 2 mPTSs, which function independently of Pex19p [39]. Nevertheless, we also cannot exclude the possibility that for some class 2 mPTS-containing proteins, targeting may be mediated by virtue of an association with bona fide class 1 mPTS-containing proteins.

3.4. mPTS consensus motifs

One major challenge in the field is to find a consensus sequence that resembles a prototypic mPTS. Until now, several consensus motifs have been postulated. The first, x(6,21)–[KR]–[KR]–x(3,8)–[TS]–x(2)–[DE]–x, was obtained by sequence comparison of the short hydrophilic loop of CbPMP47, which was first claimed to be its mPTS, with other peroxisomal membrane proteins including HsPMP34, ScAnt1p, RnPMP70, Podospora anserina (Pa) Pex2p, ScPex3p, and Hansenula polymorpha (Hp) Pex10p [51]. However, as already discussed above, (i) this loop by itself is targeted to peroxisomes only very poorly [52], and (ii) the more recently identified mPTSs of Pex3p, PMP34, and PMP70 do not contain the proposed consensus motif (Tables 1.4). Next, by using the experimentally determined mPTSs of CbPMP47, ScPex15p, ScPex3p, PpPex3p, and HpPex3p, another motif ([RK]–x–[RK]–x–[RK]–x–[L]–x0,10)–[FY] was reported [27]. This motif is characterized by the presence of several positively charged amino acids and is located on the luminal side of the membrane, close to a predicted transmembrane sequence [27]. More recently, a comparison of PMP22 orthologues revealed the presence of a conserved motif, Y–x2,3–L–x3–P–x3–[KQNM], which is sufficient to target a polypeptide containing two transmembrane segments to peroxisomes [44]. Interestingly, this motif is present in the cytoplasmic N-terminal tail of HsPMP22 and is enclosed by another motif ([KR]–x6,9–x–[LFI]–x–[KR]–x3–[LFI]) that has been identified in the mPTSs of HsPex2p and HsPMP32 [32]. Finally, it has been reported that the mPTSs of the ABC transporters HsALDP, HsPMP69, and ScPxa1p are characterized by the presence of the sequence motif F–[FL]–x–[RQK]–x–[IL]–x–[LS]–x–[KR]–x–[VIL]–x–[FIV]–x–[P] [56]. As many of the currently identified targeting elements retain interaction with Pex19p (see above), it is interesting to note here that the Pex19p-binding sites resemble short, linear, probably helical peptides with a minimal length of 11 amino acids which contain both basic and hydrophobic residues [38]. Finally, a yeast-based algorithm (x(3)–ACFILQVWY–x(3)–ACFILQVWY–x(3)–[CFILTVW]–x(3)–[CFILTVW]–x(3)–[CFILTVW]–x(3)) that scans for putative Pex19p-binding sites was developed [38]. However, although this algorithm has already been successfully used to predict true Pex19p-binding sites in PMPs from yeast [38], man [61], and Trypanosoma brucei [57], it should be noted that this approach is prone to high rates of false positive predictions [38].

3.5. Multiple mPTSs in a single PMP

The fact that several multistripping PMPs have acquired multiple mPTSs (Tables 3 and 4), suggests that this is a biologically relevant event presumably reflective of their import mechanism. Indeed, it has been proposed that multiple sorting signals act cooperatively to ensure efficient membrane localization of PMPs [34]. In this regard, it should be noted that (i) many mPTSs contain a Pex19p-binding site (see above), and (ii) Pex19p binds and stabilizes newly synthesized PMPs in the cytoplasm [39, 62]. Therefore, multiple mPTSs within one PMP might serve to bind multiple Pex19p molecules which in turn would result in the shielding of the hydrophobic regions in newly synthesized PMPs from the cytoplasm, preventing deleterious effects on protein folding and solubility [39]. Others have suggested that multiple mPTSs within a single protein might function in parallel to accommodate differences in targeting pathways among peroxisome populations [53]. The nature of these various pathways is unknown.

4. Evolutionary conservation

As can be inferred from studies in which heterologous expression systems are used, targeting of peroxisomal membrane proteins is conserved between different species of mammals [19, 31, 34, 45, 55], plants [2, 6, 11] and yeasts [63, 64]. In addition, Landgraf and co-workers [56] have shown that ScPex13p can be targeted to mammalian peroxisomes and others were able to demonstrate that the mPTS of HsALDP is functional in yeast [61]. These authors also showed that the Pex19p-binding site of HsALDP is interchangeable with that of ScPex13p in an in vivo targeting assay. Finally, PMP targeting has also been conserved between trypanosomes and mammals. Indeed, the mPTSs of both TbGAT1 and TbGAT2 bind to...
HsPex19p and are sufficient to direct a reporter protein to peroxisomes in mammalian cells [57]. Summarized, these findings show that the PMP targeting mechanism has been conserved throughout evolution.

5. Conclusions and perspectives

At present, there is convincing experimental evidence to support the following key ideas: (i) PMPs can be sorted directly from the cytoplasm to peroxisomes or travel to peroxisomes via the ER [1,3,4]; (ii) mPTSs may overlap with ER targeting signals or contain targeting information that is also recognized by the mitochondrial protein import machinery [27,37]; (iii) PMPs may contain one single or multiple mPTSs [34,39]; (iv) most, but not all, mPTSs contain a cluster of basic amino acids; and (v) Pex19p is essential for the targeting of many, but not all, PMPs to the peroxisomal membrane [42]. Taking these elements into account, it is very unlikely that a universal mPTS consensus sequence will ever be identified. However, it is becoming increasingly clear that mPTSs consist of at least two functionally distinct domains: (i) a targeting element, which directs the newly synthesized PMP from the cytoplasm to its target membrane, and (ii) an adjacent hydrophobic segment, which is required for the permanent insertion of the protein into the peroxisomal membrane [38]. In general, the complexity of the mPTSs is proportional to that of the PMPs containing them (Tables 1–4). An eventual goal may be to identify “class-specific” mPTS consensus sequences. Indeed, it is very likely that PMPs that are imported in a mechanistically similar way share the same type of mPTS. However, to accomplish this task, more complete and accurate experimental data are needed. For example, at present many inconsistent reports exist regarding the topology of certain PMPs (see above). In addition, for PMPs that have been shown to travel to peroxisomes via the ER, it is not clear whether these proteins are also able to travel directly to the membrane of mature peroxisomes. Finally, as the transit of some PMPs through the ER may be too rapid for detection, one can currently not exclude the possibility that the PMPs considered to be targeted via the cytoplasm-to-peroxisome pathway, also pass through the ER on their way to peroxisomes.

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