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Review

PTS2 Co-receptors: Diverse proteins with common features

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Abstract

One feature of the PTS2 import pathway is the separation of the roles of the PTS receptor between two proteins. Pex7p alone is insufficient to act as the receptor for the import cycle for peroxisomal matrix proteins. In all cases, Pex7p needs a PTS2 co-receptor to form an import-competent PTS2 receptor complex together with the PTS2 cargo. We provide an overview of the proteins that have been identified as PTS2 co-receptors and discuss their proposed functions.

Keywords: Peroxisome; Protein import; PTS2-receptor; Pex5p; Co-receptor

Proteins that reside in the peroxisomal matrix are post-translationally imported into the peroxisome. These matrix proteins contain one of two well-characterized peroxisomal targeting signals, PTS1 and PTS2, that are specifically recognized by their soluble receptors, Pex5p and Pex7p, respectively (for details see Subramani, this BBA issue). The PTS2 import pathway shares the main features of the Pex5p-mediated import of PTS1 proteins. First, the PTS2 receptor, Pex7p, interacts with folded and even oligomeric cargo proteins [1–3]. Second, cargo-loaded PTS2-receptor binds to the membrane peroxins Pex13p [4] and Pex14p [5,6], both of which are also required for the docking of Pex5p at the peroxisomal membrane. Third, both import pathways depend strongly on the presence of the same set of membrane-bound peroxins. These include, in addition to Pex13p and Pex14p, Pex17p as another component of the yeast docking complex, the RING-finger peroxins Pex2p, Pex10p, Pex12p, the UBC-conjugating enzyme Pex4p and its membrane anchor Pex22p and, at least in yeast, Pex8p, which links the docking complex to the RING-finger complex [7] (for a recent review see [8]). Fourth, after delivery of its cargo-proteins, Pex7p is released to the cytosol for another round of import [9]. The import process is strongly dependent on ATP [1], suggesting that the PTS2 receptor, like the PTS1 receptor Pex5p, uses the same export machinery, including the ATPases Pex1p and Pex6p and their membrane anchor Pex15p/Pex26p. These findings have led to the concept of the receptor cycle for both the PTS1 and PTS2 receptors (for details see Subramani, this BBA issue).

However, unlike the PTS1 receptor Pex5p, the PTS2 receptor Pex7p is necessary, but not sufficient, to carry out all steps of the receptor cycle. In contrast to the original assumption reflected in the term PTS2 receptor, Pex7p always requires additional soluble proteins to be fully active. These Pex7p-binding partners were discovered after the identification of Pex7p and are described in the literature as “helper”, “assistant”, “auxiliary” or “accessory” proteins of PTS2-import. These terms suggest that these proteins play only a minor role in the PTS2 import pathway. In contrast, this review will discuss recent data that support the proposition that the soluble Pex7p-binding proteins fulfil key functions in the import of PTS2 proteins. Therefore, we propose the term PTS2 co-receptors for this group of peroxins.

1. Diversity of the PTS2 co-receptors

While Pex7p is well conserved among eukaryotic organisms, the PTS2 co-receptors comprise a group of species-specific proteins that includes Pex5p, Pex18p, Pex20p and Pex21p. The

Abbreviations: AAA, ATPases associated with various cellular functions; Pex7p-BD, Pex7p-Binding Domain; Pex, peroxin; PTS, peroxisome targeting signal; TPR, tetratricopeptide repeat

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first PTS2 co-receptors, Pex18p and Pex21p, were described in *Saccharomyces cerevisiae* in 1998 by Purdue and Lazarow [10]. While the majority of yeast peroxins were identified by functional complementation of mutant strains defective in peroxisome biogenesis, these two peroxins were found by two-hybrid library screening due to their ability to bind Pex7p. The finding that both proteins are functionally redundant explains why they were missed in genetic screens. Whereas single deletion mutants of *PEX18* and *PEX21* are partially affected in PTS2-mediated import, the double mutant *pex18*Δ*pex21*Δ exhibits a typical *pex* phenotype. Like Pex7p, both peroxins are largely cytosolic, with only a minor fraction found associated with peroxisomes. Despite their redundancy in function, they display only an overall sequence identity of 23% at the amino acid level.

At the same time that Pex18p and Pex21p were discovered, Titorenko and coworkers reported the complementation of a *pex* mutant of the yeast *Yarrowia lipolytica* defective in PTS2-mediated import [11]. Besides its dual location in the cytosol and with peroxisomes, Pex20p had seemingly not much in common with Pex18p and Pex21p. The proteins display a low overall sequence similarity, Pex7p as a possible binding partner was not identified in this yeast at the time, and, most importantly, Pex20p was shown to bind the PTS2 protein thiolase and to be required for its targeting to the peroxisome. These observations implied that Pex20p may fulfil the function of not only Pex18p/Pex21p but also of Pex7p in *Y. lipolytica*.

However, subsequent studies challenged this view and demonstrated that Pex18p/Pex21p and Pex20p share a common function. Einwächter and colleagues showed that the expression of *Yl*Pex20p in a *S. cerevisiae pex18*Δ*pex21*Δ double knockout strain could partially rescue the defective PTS2 import pathway [12]. After the identification of Pex20p in *Neurospora crassa*, the same experiment was successfully repeated with *Nc*Pex20p [13]. During the last year, two additional Pex20p homologues were reported from *Hansenula polymorpha* [14] and *Pichia pastoris* [15]. Interestingly, the genomes of all these four fungi, *Y. lipolytica*, *N. crassa*, *H. polymorpha*, and *P. pastoris*, contain a *PEX7* homologue, and in *N. crassa* and *P. pastoris*, even the PTS2 receptor Pex7p was described [13,16]. These data indicated that, in most lower eukaryotes, the PTS2 co-receptor is Pex20p and that Pex18p and Pex21p in *S. cerevisiae* were exceptions to the rule. However, a recent genomic search revealed the existence of genes for both Pex18p and Pex21p also in *Candida glabrata* (see below).

In higher eukaryotes, homologues of the fungal PTS2 co-receptors have not been found. Moreover, in 1998 the puzzling observation was made that the mammalian PTS1 receptor is required for the import of PTS2 proteins [17,18]. Today, it is well established that mammalian cells generate two isoforms of Pex5p through alternative splicing, Pex5pS and Pex5pL, and, as was shown recently in Chinese hamster ovary cells, an additional form, Pex5pM [19]. Only one isoform, Pex5pL, has been established to be involved in PTS2 protein import and physically interacts with the PTS2 receptor, Pex7p [20,21]. The same Pex7p binding property is displayed by the PTS1 receptor of plants. The *PEX5* gene of *Arabidopsis thaliana* expresses only one transcript, and its gene product, *At*Pex5p, resembles the long form of mammalian Pex5p [22].

These findings in higher eukaryotes suggested for the first time that distinct PTS1 receptors could act as co-receptors of Pex7p in the PTS2 import pathway. This in turn provided an explanation why no orthologues of the fungal PTS2 co-receptors have been discovered in these two eukaryotic kingdoms.

An interesting question related to Pex18p and Pex21p is why *S. cerevisiae,* and perhaps *C. glabrata*, possesses two partially redundant PTS2 co-receptors. One possibility would be that their gene expression is differently regulated to ensure that the capacity of the PTS2 import pathway can be adjusted to variable physiological conditions. Known properties of *Sc*Pex18p and *Sc*Pex21p are consistent with such a possibility. When fatty acids and not glucose are the sole source of carbon and energy, the expression of the main PTS2 protein thiolase in *S. cerevisiae* is upregulated 50-fold as compared to the other β-oxidation enzymes whose genes are controlled by an oleic acid-inducible element in their promoters (for details see Rottensteiner, this BBA issue). A similar transcription profile (glucose-repressed and oleic acid-induced) has been reported for *Sc*Pex18p [23]. As Pex18p is an extremely short-lived protein with a half-life of approximately 10 min [24], these features together would allow for a fast adjustment of the steady state expression level of this peroxin.

In contrast, the gene for *Sc*Pex21p seems to lack an oleic acid-inducible promoter, suggesting that *Sc*Pex21p could be involved in processes other than β-oxidation. Furthermore, *Sc*Pex21p has been reported to interact specifically with seryl-tRNA synthetase [25]. A systematic mutant analysis revealed that Pex21p may be required for proper meiosis [26]. How these processes are related to each other, if at all, or to peroxisome biogenesis is an open question for future studies.

2. Common structural features of PTS2 co-receptors

In addition to a common function, the PTS2 co-receptors share structural similarities. Despite the low overall similarity between the primary sequences of Pex18p, Pex20p, Pex21p and the N-terminal half of HsPex5pL, their sequence alignment reveals three conserved regions (Fig. 1). These are the Pex7p binding domain (Pex7p-BD), a domain of about 30 amino acyl residues close to the N-terminus, and one or more WxxxF/Y motifs (Fig. 1).

The highly conserved core region of the common Pex7p-BD comprises 20 to 30 amino acyl residues that most likely form an amphipathic α-helix. In human Pex5p, this domain exists only in the long isoform of the PTS1 receptor, Pex5pL. Pex5pL contains a 37-amino acid long insert (amino acyl residues 215 to 251) that is positioned between amino acids 214 and 215 of Pex5pS and is encoded by an extra exon. Dodt and colleagues showed that amino acids 1–230 of Pex5pL are required for PTS2 protein import, and amino acids 191 to 222 are sufficient for the binding of *Hs*Pex7p [27]. The same authors identified a 21-amino acid long peptide motif of Pex5pL, amino acids 209 to 229, that overlaps with the region sufficient for full PTS2

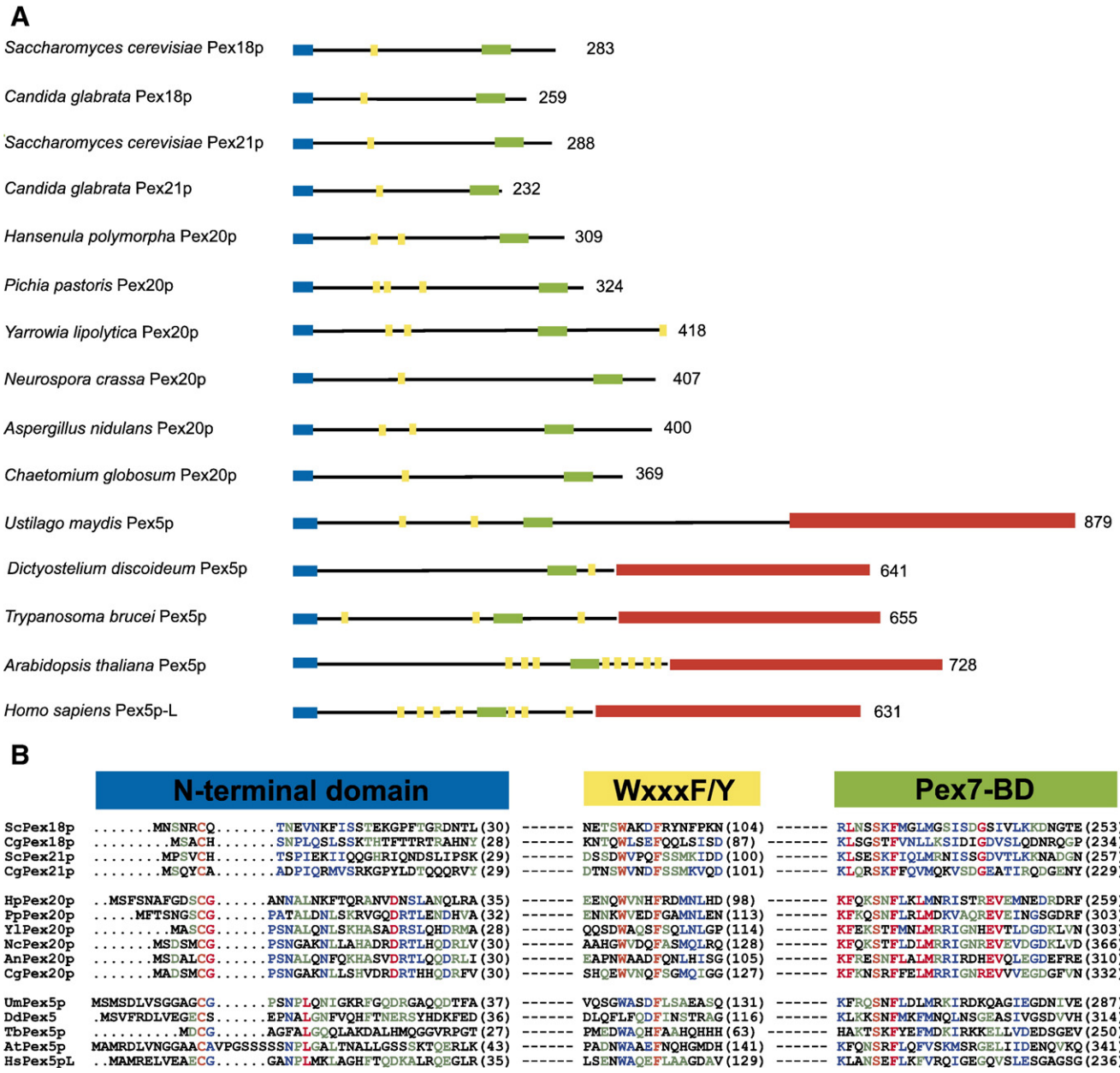

Fig. 1. Conserved regions of PTS2 co-receptors. (A) Schematic representation of selected PTS2 co-receptors. Conserved sequence regions are the N-terminal domain (blue boxes), one or more WxxxF/Y-motifs (yellow boxes), the Pex7p-binding domain (green boxes), and a TPR-rich domain within the Pex5p subgroup (red boxes). (B) Sequence comparison of the conserved amino acid regions of selected PTS2 co-receptors. The N-terminal domain, the first WxxxF/Y motif and the Pex7-BD of *Saccharomyces cerevisiae* Pex18p (*Sc*Pex18p, UniProtKB/Swissprot primary accession number P38855), *Candida glabrata* Pex18p (*Cg*Pex18p, UniProtKB/TREMBL entry accession number Q6FR00), *Saccharomyces cerevisiae* Pex21p (*Sc*Pex21p, P50091), *Candida glabrata* Pex21p (*Cg*Pex21p, Q6FTX7), *Hansenula polymorpha* Pex20p (*Hp*Pex20p, Q3ZJZ2), *Pichia pastoris* Pex20p (*Pp*Pex20p, Q2VUH8), *Yarrowia lipolytica* Pex20p (*Yl*Pex20p, O74211), *Neurospora crassa* Pex20p (*Nc*Pex20p, Q8J1Y9), *Aspergillus nidulans* Pex20p (*An*Pex20p, Q5B562), *Chaetomium globosum* Pex20p (*Cg*Pex20p, Q2GMU4), *Ustilago maydis* Pex5p (*Um*Pex5p, Q4P464), *Dictyostelium discoideum* Pex5p (*Dd*Pex5p, Q54MD1), *Trypanosoma brucei* Pex5p (*Tb*Pex5p, Q57W55), *Arabidopsis thaliana* Pex5p (*At*Pex5p, O82467), *Homo sapiens* Pex5p long isoform (*Hs*Pex5p-L, Q96FN7) are shown. Red coloured letters indicate that these amino acyl residues are completely conserved within each PTS2 co-receptor subgroup. Blue coloured letters denote at least 75% sequence identity or strong similarity within each PTS2 co-receptor subgroup. Green colour marks amino acyl residues representing more than 50% sequence identity or similarity. Numbers in brackets indicate the sequence position of the C-terminal amino acyl residues within each of the three conserved regions.

rescue activity and Pex7p interaction and is shared by *S. cerevisiae* Pex18p/Pex21p. Genetic evidence for a specific Pex7p binding site in Pex5p came from the identification of two interesting *pex5* mutants. A mutation in mammalian Pex5p of a highly conserved serine, S214F, was found to cause profound defects in PTS2 import [20]. In *Arabidopsis thaliana*, the *pex5-1* mutant with a substitution of serine 318 by leucine was defective in the import of PTS2 proteins but not PTS1 proteins [22]. Mutagenesis studies of the corresponding amino acid within the sequences of Pex18p, Pex21p and *Y. lipolytica* Pex20p also resulted in an impairment of Pex7p binding [12]. Taken together, these data clearly demonstrated the existence of

a conserved Pex7p binding site in the different members of the co-receptor group, and the functional importance of this common structural element.

To exploit the wealth of available genomic data, we screened the UniProtKnowledgebase (Swissprot/TREMBL) database, release 8.1, with the consensus sequence of the Pex7p-BD derived from available PTS2 co-receptor sequences, as indicated in the legend to Fig. 1. It resulted in 54 matches among those 32 eukaryotic proteins sharing overall sequence similarity with Pex5p, Pex18p, Pex20p or Pex21p and belonging to a large variety of different eukaryotic organisms (unpublished results) (Table 1). Although the expression and function of several of these proteins remain to be established, the identification of potential new PTS2 co-receptors in particular species might be useful in extending our view of the phylogenetic classification of this class of proteins. In this context, it is interesting to note that the genome of the pathogenic yeast *C. glabrata* contains genes coding for both Pex18p and Pex21p (Fig. 1). This indicates that these two peroxins of *S. cerevisiae* (and other species of this genus) might lose their exceptional status among the PTS2 co-receptors. It is also noteworthy that the filamentous ascomycete *Ashbya gossypii* which had not undergone whole-genome duplication during evolution like *S. cerevisiae* and other yeasts [28] seem to possess a homologous *PEX21* gene whereas an additional *PEX18* related gene could not be detected. This is even more remarkable since all other peroxins known to be involved in yeast peroxisomal protein import are also present in Ashbya (data not shown; for more details see http://agd.unibas.ch). These findings suggest that *PEX18* arose as a result of gene duplication of an ancient *PEX21* gene and thereby, clearly support the view that both PTS2 co-receptors in *Saccharomyces spec*. and *Candida glabrata* represent true paralogs. Another unexpected result from our *in silico*- analysis was that the genomes of two heterobasidiomycetes, the fungal plant pathogen *Ustilago maydis* and the human pathogen *Cryptococcus neoformans*, contain genes encoding a PTS1 receptor with a Pex7p-BD binding site (Fig. 1).The absence of *PEX18*, *PEX20* and *PEX21* genes in these organisms further supports the assumption that the PTS1 receptor, Pex5p, has acquired the function to act as a PTS2 co-receptor not only in mammals and plants but also in fungi. Only in ascomycetes like yeasts and *N. crassa* do other peroxins fulfil this role. The observation that the PTS1-receptor carries a Pex7-BD in these fungi and several primitive eukaryotes like *Trypansosoma*, *Leishmania* and the slime-mold *Dictyostelium* (Table 1 and Fig. 1) suggests that the two PTS import pathways have converged more anciently than was previously thought.

Very little is known about the second common structural element of the PTS2 co-receptors, the conserved domain of about 30 amino acid residues at their N-termini. This domain was first recognized in a sequence comparison between *Yl*-Pex20p and all available Pex5p-sequences (including those without Pex7p-BD) [11]. An important functional role for these regions was suggested by Dodt and colleagues who demonstrated that a deletion of the N-terminal 49 amino acids residues in human Pex5pL results in a PTS2 import defect [27]. The most striking residue within this domain is a conserved cysteine. It is interesting to note that in most, if not in all, Pex5p sequences, this is the only cysteine present in the whole N-terminal part positioned in front of the recognition domains for PTS1 proteins or the PTS2 receptor. However, an explicit function for this residue has not yet been elucidated. Surprisingly, deletion of the N-terminal 16 amino acids of

Table 1
Species-specific distribution of proteins containing the Pex7p-BD consensus sequence

	Fungi	Protista	Plants	Animals
Pex18p	*Saccharomyces cerevisiae*			
	Candida glabrata			
Pex21p	*Saccharomyces cerevisiae*			
	Candida glabrata			
	Ashbya gossypii			
Pex20p	*Yarrowia lipolytica*			
	Pichia pastoris			
	Hansenula polymorpha			
	Debaryomyces hansenii			
	Chaetomium globosum			
	Aspergillus several species			
	Neurospora crassa			
Pex5p	*Ustilago maydis*	*Trypanosoma cruzi*	*Citrullus lanatus*	*Homo sapiens*
	Cryptococcus neoformans	*Trypanosoma brucei*	*Nicotiana tabacum*	*Cricetulus griseus*
		Leishmania major	*Arabidopsis thaliana*	*Mus musculus*
		Leishmania donovani	*Brassica napus*	*Cavia porcellus*
	Dictyostelium discoideum		*Oryza sativa*	*Gallus gallus*
				Bos taurus

The sequence pattern which was used to identify novel eukaryotic proteins containing a Pex7-BD was [KRH]-[YVMIFLA]-[QSAKETNRDG]-[QSAKETNRDG] -S-[ETKRN]-F-[YVMIFL]-[QSAKETNRDG]-[YVMIFL]-[YVMIFL]-[QSAKETNRDG]-[QSAKETNRDG]-[YVMIFL]. The pattern search was performed by ScanProsite (www.expasy.ch/tools/scanprosite) on the UniProtKB (SwissProt release 50.1 /Trembl release 33.1) database, release 8.1, using a filter for eukaryotic proteins only. The original scan revealed 54 matches of which 32 full-length proteins either were notified earlier as, or shared significant structural similarities with, known PTS2 co-receptors.

*Pp*Pex20p containing the conserved cysteine did not impair peroxisomal protein import, whereas truncation of 19 residues resulted in a *pex* phenotype [15]. It would be interesting to see whether the efficiency of PTS2-mediated protein import is affected in *P. pastoris* mutant cells expressing the Pex20p form lacking the first 16 amino acids.

Different numbers of the third common structural element, the WxxxF/Y-motif or di-aromatic pentapeptide repeats, are found in all known PTS2 co-receptors and also in Pex5p isoforms that do not bind Pex7p and therefore do not belong to this group of peroxins. These motifs were originally defined in the sequence of the human PTS1 receptor to serve as ligands for Pex14p, one component of the docking complex at the peroxisomal membrane [29]. Later studies in other organisms confirmed that Pex5p can bind these repeats in Pex14p. Pex5p binds via WxxxF/Y repeats to Pex14p in *A. thaliana* [30] and *Trypanosoma brucei* [31] and *Pp*Pex20p interacts with *Pp*Pex14p through one of its three WxxxF/Y-motifs [15]. This interaction was shown for the first time in *P. pastoris* to be actually involved in the docking step of a fungal PTS2 co-receptor.

However, during the last years it has become known that not all WxxxF/Y motifs in Pex5p serve as Pex14p-ligands (for details see Azevedo and Schliebs, this BBA issue). While some of them function as binding sites for Pex13p, no binding proteins could yet be identified for others. Pex13p was found to interact with *Hs*Pex5p [32], *Yl*Pex20p and *Sc*Pex21p [12] but whether all these interactions occur directly via WxxxF/Y motifs as shown for the human PTS1 receptor remains to be established.

3. Possible functions of PTS2 co-receptors

According to the receptor cycle, the cytosolic import receptors for matrix proteins have to carry out steps that lead to their membrane-bound state (cargo-binding and docking) and subsequent steps in/at the peroxisomal membrane that remain ill-defined (translocation and return). On the basis of immunoprecipitation studies in various organisms [10,33], there is general agreement that the PTS2 receptor, PTS2 co-receptor and PTS2 cargo form a cytosolic complex that acts as the import-competent receptor. However, why Pex7p is insufficient and requires a co-receptor is not completely known. Central questions of the PTS2 import pathways, which of the various receptor tasks are assigned to Pex7p and which to the co-receptor, are still a matter of debate. Accumulating data suggest a number of different possibilities that are discussed below.

3.1. PTS2 co-receptors as targeting modules of the PTS2 receptor complex

*Sc*Pex18p and *Sc*Pex21p were the first PTS2 co-receptors shown to be required for the targeting of Pex7p to the peroxisome [10]. Purdue and colleagues demonstrated that the recognition of PTS2 by Pex7p does not require Pex18p/Pex21p, whereas the latter proteins interact with the PTS2 cargo thiolase only in the presence of Pex7p. Moreover, in the absence of Pex18p and Pex21p, epitope-tagged Pex7p is completely mislocalized to the cytosol. A functional relationship between Pex7p and Pex20p was also reported for *N. cra*ssa [13] and recently for *P. pastoris* [15].

What is the situation in higher eukaryotes? There, the PTS1 receptor Pex5p acts as the PTS2 co-receptor (see above). In addition to its Pex7p-binding domain, this peroxin comprises two other distinctly different functional parts. While its C-terminal half with six or seven TPR repeats recognizes PTS1 cargo proteins, the N-terminal half has been implicated in other steps of the receptor cycle, including targeting and docking at the peroxisomal membrane [34,35]. Dodt and co-workers could show that the N-terminal 214 amino acids are sufficient to direct the Pex7p–cargo complex to the peroxisome [27].

Exploiting the ability of the N-terminal half of *Sc*Pex5p (Pex5p-N) to bind acyl-CoA oxidase, a non-PTS1, non-PTS2 cargo protein in *S. cerevisiae*, Schäfer and colleagues demonstrated that this part of Pex5p is sufficient to facilitate the import of this major peroxisomal matrix protein [35]. Moreover, the authors found that *Sc*Pex18p, when fused to the PTS1 binding domain of *Sc*Pex5p, at least partially replaces Pex5p-N in PTS1 import. Extrapolating from these data, these authors suggested a model for the PTS2 import pathway in which *Sc*Pex18p is predicted to be the targeting module and *Sc*Pex7p the subunit of the PTS2 receptor complex that specifically binds the PTS cargo proteins (Fig. 2). The functional similarity between Pex5p-N and Pex18p provides a rationale for the intriguing observation that higher eukaryotes do not possess Pex18p, Pex20p or Pex21p (see Table 1) but require only the PTS1-receptor containing a Pex7-BD (Fig. 2).

Assigning the targeting function of the PTS2 receptor complex exclusively to the PTS2 co-receptor subunits may be

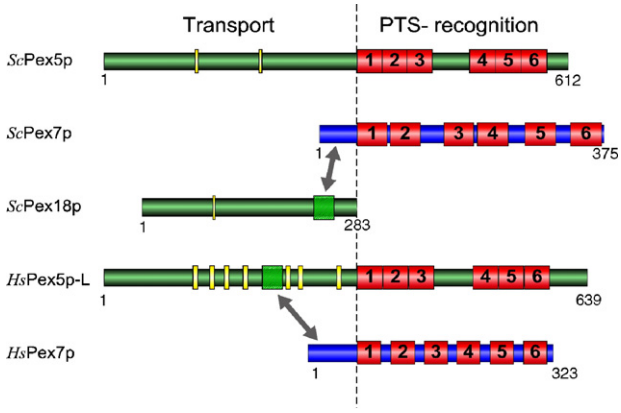

Fig. 2. Structural and functional similarities between PTS1 and PTS2 receptor systems of human and yeast. Whereas PTS1-mediated protein import requires only Pex5p, the fully functional PTS2 receptor comprises a heteromeric complex consisting of the PTS2-recognizing Pex7p and a co-receptor like Pex18p (or Pex21p) in *S. cerevisiae* or Pex5p-L in human. PTS2 receptor complex formation is mediated by a Pex7p-binding domain (dark green box) conserved in all co-receptors and a not yet mapped binding site on Pex7p. PTS2 co-receptors are required for the targeting of the cargo–receptor complex to the peroxisome and are probably involved in other membrane-bound steps of the receptor cycle (for further details see text). Yellow bars indicate WxxxF/Y motifs. Red boxes within the PTS recognition sites of Pex5p and Pex7p represent TPR-motifs and WD-40 motifs, respectively.

an oversimplification. There are at least two exceptions that demonstrate that Pex7p in several organisms does not require a targeting module. Leon and colleagues showed in *P. pastoris* that the association of both Pex7p and Pex20p with the peroxisomal docking subcomplex can be independent of the other protein and that each peroxin does not significantly affect the peroxisomal localization of the other [15]. Moreover, Stein and co-workers showed by yeast two-hybrid analysis, co-immunoprecipitation and *in vitro* assays that Pex7p in *S. cerevisiae* interacts with Pex13p/Pex14p/Pex17p, irregardless of the presence of Pex18p and/or Pex21p [6]. How these findings in *S. cerevisiae* can be reconciled with the original observation by Purdue and Lazarow that Pex7p is mislocalized to the cytosol in *pex18Δpex21Δ* cells is not yet clear. A reasonable possibility is that Pex7p still requires PTS2 co-receptor-dependent targeting to the membrane (for example a tethering step) before it actually associates with the docking proteins.

3.2. PTS2 co-receptors as enhancers of cargo binding by Pex7p

Another possible explanation for the fact that PTS2 co-receptors are required to confer import competence to the Pex7p/PTS2 cargo complex is that they might stabilize the Pex7p–cargo complex. There are several ways how this could be accomplished. First, PTS2 co-receptors could act as molecular chaperones for cargo proteins and thereby stabilize a conformation of the cargo that makes its PTS2 more accessible to Pex7p, as suggested by Titorenko and colleagues. [11]. In fact, these authors reported that *Yl*Pex20p is required for oligomerization of the PTS2-protein thiolase, which is a prerequisite for its targeting to the peroxisome. Second, PTS2 co-receptors could assist the folding of the PTS2-receptor itself. This would be in line with our unpublished observation that when overproduced, Pex7p becomes insoluble in *S. cerevisiae*. This might indicate that a folding-assisting factor, *i.e.* another subunit of the PTS2–receptor complex, is not present in the necessary amounts. A third scenario is that PTS2 co-receptors would allosterically increase the sensitivity of the PTS2-receptor to its cargo by binding to other sites of the Pex7p–cargo complex. Such a model would be in line with findings by Titorenko and colleagues showing that the association of *Yl*-Pex20p with thiolase is independent of the PTS2 in the cargo protein. Although these described observations are consistent with the proposition that yeast PTS2 co-receptors increase the efficiency of cargo binding by Pex7p, direct evidence for such a model is still lacking.

3.3. Membrane-bound PTS2 co-receptors

Accumulating data suggest that the PTS2 co-receptors play essential roles not only in the formation and targeting of receptor–cargo complexes but also in later steps of the receptor cycle in/at the peroxisomal membrane. In this respect, there are also striking similarities between the PTS2 co-receptors and the PTS1 receptor Pex5p.

First, the fungal PTS2 co-receptors display the same dual localization between the cytosol and peroxisomes as has been found for the two PTS import receptors, Pex5p and Pex7p. Moreover, at least that fraction of Pex5p that is peroxisomal displayed properties characteristic of integral proteins (see Azevedo and Schliebs, this BBA issue) In contrast, there is no evidence that the PTS2 receptor Pex7p behaves as an intrinsic protein. Second, Pex20p from *Y. lipolytica* and *P. pastoris* interacts with the intraperoxisomal peroxin Pex8p, as does Pex5p, indicating that both Pex20p and Pex5p reach the inside of the peroxisome during a receptor cycle. Third, *Sc*Pex18p and *Pp*Pex20p recycle back to the cytosol and are subject to polyubiquitination like Pex5p. The absence of peroxins involved in late steps of the import cycle (the ubiquitin-conjugating peroxin Pex4p and the AAA-peroxins Pex1p and Pex6p) cause a mostly peroxisomal localization of all three peroxins. In *P. pastoris*, this phenotype requires in addition the Pex20p-K19R mutation preventing ubiquitin-dependent degradation of Pex20p. There is one notable difference in the fates of both fungal PTS2 co-receptors after their release from the peroxisome. While *Sc*Pex18p was shown to be constitutively degraded by the ubiquitin/proteasome pathway resulting in a short half-life of about 10 min, *Pp*Pex20p has been proposed to re-enter peroxisomes in a subsequent round of import and is degraded by a ubiqutin-dependent quality-control mechanism triggered only in the absence of recycling.

Finally, a study that most clearly, but still indirectly, demonstrates the functional equivalence of *Sc*Pex18p and the N-terminal half of *Sc*Pex5p used a chimeric peroxin in which the PTS2 co-receptor *Sc*Pex18p (without its Pex7p binding site) was fused to the C-terminal PTS1 recognition site of Pex5p. This artificial protein was able to partially complement the PTS1 import defect in a *PEX5* deletion strain. On the basis of these data, Schäfer and colleagues concluded that both Pex18p and Pex5p-N are capable of translocating cytosolic proteins into the peroxisome.

Taken together, all the structural and functional similarities suggest that the fungal PTS2 co-receptors act as functional counterparts of Pex5p-N in peroxisomal protein import (Fig. 2). However, the different functions of a peroxisomal import receptor (cargo-recognition and transport) appear not to be as strictly separated in the Pex7p/fungal PTS2 co-receptor pairs as in the two halves of Pex5p. The known exceptions are the interaction of Pex7p with Pex14p and Pex13p (see above) and the recognition of the PTS2 of Pex8p by *Pp*Pex20p (see below).

3.4. Pex20p as an import receptor in its own right

The notion that PTS2 co-receptors possess properties necessary for the function of import receptors in the peroxisomal receptor cycle leads to the question whether the fungal PTS2 co-receptors can replace Pex7p as cargo binding proteins.

Such an example was first reported from studies in *Y. lipolytica* in which an interaction between *Yl* Pex20p and the PTS2 cargo protein thiolase was detected under *in vivo* and *in vitro* conditions using co-immunoprecipitation and overlay

techniques [11]. However, the involvement of Pex7p in this interaction was not rigorously excluded because, at the time, *Yl*Pex7p was not identified. The fact that the detected binding between *Yl*Pex20p and thiolase was not dependent on the PTS2 of this enzyme, and that *Yl*Pex20p contains a Pex7p-binding domain, also argue for a re-investigation of this interaction.

Very recently, two other studies addressed this question again and provided convincing evidence that Pex20p of *H. polymorpha* and *P. pastoris* can specifically recognize a PTS2. *Hp*Pex20p has been reported to bind *in vitro* a synthetic peptide containing a PTS2 sequence [14]. This conclusion was based on fluorescence correlation spectroscopy experiments performed in the absence of Pex7p with a purified, oligomeric form of Pex20p. A study in *P. pastoris* demonstrated that *Pp*Pex20 binds Pex8p in a PTS2-dependent manner [36]. Mutation in the putative PTS2 motif of Pex8p abolished its interaction with Pex20p and its Pex20p-dependent import into peroxisomes. This is the first clear evidence that the PTS2 motif on Pex8p is necessary for its peroxisomal import in the context of the full-length protein. Apparently contradicting this, *Yl*Pex8p fused at its C-terminus with the hemagglutinin (HA) tag was found in the organelle pellet fraction in *Y. lipolytica pex20* knockout cells. However, on the basis of the presented data, it is not possible to exclude that *Yl*Pex8p reached the peroxisome by an alternative pathway or was on the cytosolic side of the peroxisomal membrane. Remarkably, the Pex8p import pathway in *P. pastoris* still requires Pex7p. This, in turn, is consistent with the general observation that in all organisms tested, deletion of the *PEX7* gene abolishes PTS2 protein import.

Collectively, these data argue strongly against the possibility that Pex20p or another PTS2 co-receptor can replace Pex7p *in vivo*, but rather suggest that at least Pex20p/Pex8p might behave as a PTS2 receptor/PTS2 cargo pair whose entry into peroxisomes depends on Pex7p. At present, it seems likely that Pex8p with its unique properties (location and targeting signals) is an exception among the PTS2 cargo proteins. However, lack of relevant data do not permit an answer yet as to whether Pex8p can also be recognized in a PTS2-dependent manner by Pex18p/Pex20p.

4. Concluding remarks

Accumulating data permit the conclusion that PTS2 co-receptors are essential components of the import-competent PTS2 receptor complex. They fulfil not only functions in the assembly of this complex in the cytosol but also in membrane-bound steps of the peroxisomal import cycle. The structural and functional similarities between the PTS2 co-receptors and the PTS1 receptor Pex5p strongly support the view that the two PTS-dependent peroxisomal import pathways use the same import machinery. Initial insights have been obtained into the key issue as to how the distinct roles of the two different halves of the Pex5p molecule are distributed between the Pex7p/PTS2 co-receptor pair. It seems a reasonable assumption that all PTS2 co-receptors are actively involved in the translocation of cargo-loaded Pex7p, carrying out the same or a similar order of events. However, a final answer to this question will require especially a mechanistic understanding of the transport steps at the peroxisomal membrane.

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