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The Role of Shuttling Targeting Signal Receptors and Heat-Shock Proteins in Peroxisomal Matrix Protein Import

SEBASTIEN LEON[a] • SURESH SUBRAMANI[b]

[a]*Institut Jacques Monod-CNRS*
2 Place Jussieu
75251 Paris Cedex 05
France

[b]*Section of Molecular Biology*
Division of Biological Sciences
University of California, San Diego
La Jolla, CA 92093-0322, USA

I. Catalytic Machines Involved in Peroxisomal Matrix Protein Import

Peroxisome biogenesis requires the action of about 32 *PEX* genes encoding a family of proteins known as peroxins [1]. These are distributed in the cytosol, peroxisomal membrane, or peroxisome lumen. While a number of these proteins, particularly components of a peroxisome membrane-associated complex known as the importomer [2], regulate the entry of cargoes and cargo receptors into and out of peroxisomes, a number of proteins act catalytically to facilitate the import of proteins into the peroxisome matrix. This chapter will focus on two sets of proteins that act in this fashion.

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1. The peroxisome-targeting signal (PTS) receptors that repeatedly bind cargo in the cytosol ferry the cargo into the peroxisome lumen, and then recycle back to the cytosol for another round of import [3]. When recycling of these receptors is compromised, enzymes involved in a peroxisomal pathway known as receptor accumulation and degradation in the absence of recycling (RADAR) function to keep the peroxisome membrane clear of cargo-free receptors [3]. These will also be described briefly.
2. The Hsp70 class of proteins that undergoes cycles of ATP binding and hydrolysis.

Other enzymes required for peroxisomal matrix protein import include those, such as Pex4p, involved in protein ubiquitylation, and the AAA ATPases, Pex1 and Pex6, that associate with each other and are needed for receptor recycling from peroxisomes to the cytosol—these are reviewed separately in this volume.

II. Components Involved in Peroxisomal Matrix Protein Import

To set the stage for the discussion of these catalytic machines, we first need to understand the peroxisome biogenesis machinery itself and the general mechanism by which proteins are sorted to the peroxisome matrix.

Most proteins destined for the peroxisome matrix possess one or more PTSs. A few peroxisomal matrix proteins lack a PTS but enter the matrix simply by association with other protein partners that do have a PTS, which is a consequence of the fact that folded, oligomeric and cofactor-bound proteins can be transported into the peroxisome lumen [3]. Two classes of conserved PTSs have been described—a C-terminal tripeptide or PTS1 and an N-terminal or internal nona-peptide, named PTS2. Following the synthesis of peroxisomal matrix proteins in the cytosol, these PTSs are recognized by specific receptors/coreceptors. The Pex5 protein is the PTS1 receptor [4–7], and the Pex7 protein is the PTS2 receptor in yeasts, plants, and mammals [8–15]. However, the PTS2 pathway of import requires an auxiliary protein, or coreceptor, to transport cargoes into the matrix. In yeasts, members of the Pex20 family of proteins (Pex18 or Pex21 in *Saccharomyces cerevisiae* and Pex20 in *Neurospora crassa*, *Pichia pastoris*, and *Yarrowia lipolytica*) are also necessary for the PTS2 import pathway [16–20], whereas in plants and mammals, a long isoform of Pex5, called Pex5L, serves as the auxiliary protein for the PTS2 pathway [15, 21].

The complexes formed between cargo and the PTS coreceptors and/or receptors then interact at the peroxisomal membrane with a docking

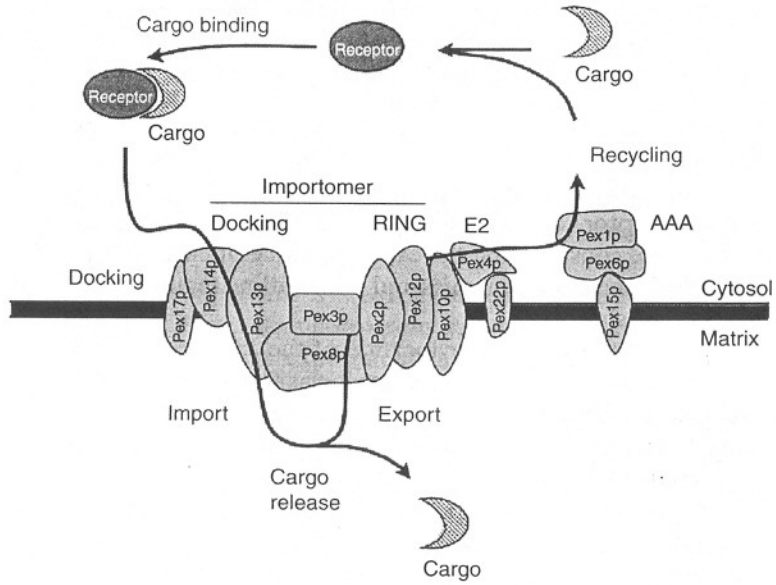

FIG. 20.1. Steps in the extended shuttle cycle of the PTS receptors during peroxisomal matrix protein import. See text for details.

subcomplex, comprised in yeasts of the peroxisomal membrane or membrane-associated peroxins, Pex3, Pex8, Pex13, and Pex14 (Figure 20.1). This docking subcomplex likely serves as the translocon for the receptor/cargo complexes [3] and associates with a second subcomplex in the peroxisome membrane, the really interesting new gene (RING) subcomplex, composed of three RING-domain proteins, Pex2, Pex10, and Pex12 [22, 23]. The docking and RING subcomplexes have been reported in different yeasts to be bridged either by Pex3 or by Pex8 to form a larger complex known as the importomer, all of whose individual constituents are necessary for import of most PTS1- and PTS2-containing proteins [22, 23].

The importomer is also in proximity, if not association, with a receptor recycling machinery [17, 24, 25], comprised in yeasts of the E2-like ubiquitin-conjugating enzyme, Pex4, anchored to the peroxisome membrane via Pex22, and two AAA ATPases, Pex1 and Pex6, that associate with the peroxisome membrane (via Pex15 in *S. cerevisiae* and PEX26 in mammals) (Figure 20.1).

There is evidence from epistasis and biochemical analyses that the docking, RING, and recycling subcomplexes act sequentially during the matrix protein import cycle [17, 26].

A. Receptor Shuttling During Peroxisomal Matrix Protein Import

The PTS receptors function in multiple rounds of cargo import into peroxisomes [24, 25, 27]. They do so by binding cargo in the cytosol, ferrying it to the peroxisome lumen, releasing cargo in the peroxisome matrix, and recycling back to the cytosol. This cycle of receptor dynamics is referred to as the extended shuttle [27, 28]. This mechanism allows each molecule of receptor to participate in several rounds of cargo import into peroxisomes. We consider first the experimental evidence for the extended shuttle model of receptor dynamics and then outline features of the specific steps in receptor shuttling.

Early work on the subcellular location of the PTS1 receptor, Pex5, was confusing because in different organisms it was found to be either cytosolic, or mostly cytosolic and partially peroxisomal, or inside peroxisomes. These could have been attributed to organism-specific variations. However, when the *S. cerevisiae* PTS2 receptor was identified by two laboratories, it was very puzzling that one group reported Pex7 to be cytosolic, while the other found it to be intraperoxisomal [10, 13, 14] (only later it was found that the predominantly intraperoxisomal localizaion of Pex7 was caused by the attachment of a C-terminal tag used to localize the protein [29]). These discrepancies suggested the possibility of a shuttling receptor [10, 30]. It was hypothesized that the use of tagged proteins to follow their subcellular location might alter the steady state distributions of these proteins among the compartments between which they shuttle.

More careful experiments with human PEX5 revealed a dual location with most of the protein being cytosolic but some was peroxisome-associated [4, 7]. The ability to shift the equilibrium between these two populations, either by modulation of conditions used for peroxisomal protein import or the use of mutants, suggested that there might be a dynamic movement of PEX5 from the cytosol to the peroxisomes. For example, under low temperature or ATP-depletion conditions that affect matrix protein import, more PEX5 was peroxisome-asssociated, and the peroxisome-associated pool was reduced on raising the temperature or on readdition of ATP [31]. Similar perturbations of the mammalian PEX5 population in the cytosol versus the peroxisomes were observed in certain *pex* mutants affecting peroxisome biogenesis [31]. Intraperoxisomal accumulation of Pex5 was also observed in the *Hansenula polymorpha pex4* mutant [32]. As a result of these data, the idea of a cycling receptor was given more credence, but these early studies assumed implicitly that the receptor ferried cargo to the peroxisomal translocon, released cargo there, and returned directly to the cytosol for another round of import (simple shuttle) [10]. Although the entry of the receptor into peroxisomes had been suggested [30], experimental data supporting this notion was lacking.

Evidence that PEX5 uses instead an extended shuttle, where it goes in and out of peroxisomes during the import cycle, came from the work of Dammai and Subramani [27]. In this study, a modified version of PEX5 was processed by a peroxisome-specific protease, before returning to the cytosol, indicating that PEX5 can sample, if not enter, the peroxisome matrix. The study also suggested that specific *cis*-acting sequences and *trans*-acting proteins may be necessary for PEX5 export from peroxisomes and recycling to the cytosol—predictions now supported by experimental data (see below).

Experiments using *in vitro* systems have addressed the requirements for the association of PEX5 with peroxisomes, as well as for its recycling to the cytosol [24, 33, 34]. These experiments also shed light on the steps in the PEX5 extended shuttle cycle. The existence of several populations of PEX5 found in these studies was correlated with different stages of the peroxisomal matrix protein import cycle [33]. PEX5 molecules in stages 0 and 1 were protease-sensitive and localized in the cytosol and to peroxisome membranes, respectively. Stage 2 defined PEX5 molecules that were peroxisome membrane-associated and rendered 2 kDa shorter at their N-termini on protease treatment, indicating that these were embedded deep in the peroxisome membrane with about 2 kDa exposed to the cytosol. Finally, stage 3 PEX5 molecules were peroxisome-associated and completely protease-resistant, suggesting they might be in the lumen. ATP-limiting conditions, which inhibit matrix protein import and receptor recycling (as indicated later), enhance the amount of stage 3 molecules. These data are compatible with a model in which PEX5 begins in the cytosol (stage 0), docks at peroxisomes (stage 1), inserts into the peroxisome membrane (stage 2), and then translocates to the preoxisome matrix (stage 3), before its final recycling to the cytosol (stage 0), for another round of import. These intermediates in the shuttling of PEX5, as well as the ability of shuttling PEX5 to participate in more than one round of import, has also been documented beautifully in an *in vitro* system capable of importing proteins into the peroxisome matrix [24].

Do the receptor and coreceptor for the PTS2 pathway also exhibit the extended shuttle? Work on *S. cerevisiae* and *P. pastoris* shows this to be true for Pex7 and Pex20, respectively [17, 29]. The N-terminal 56 amino acids of Pex7 are necessary for its peroxisomal entry [14], and fusion of green fluorescent protein (GFP) or other tags at the C-terminus slows down or inhibits Pex7 recycling from peroxisomes [29]. Further details of Pex7 behavior during the import cycle are not as clear as they are for Pex5.

The auxiliary proteins (Pex18, Pex21, or Pex20) are required for PTS2 import, but different models have been proposed for their exact functions [17, 19]. In *S. cerevisiae*, Pex18 is suggested to stabilize the PTS2 cargo/Pex7

interactions by formation of a ternary complex that then docks at the peroxisome [19]. In *P. pastoris*, Pex7 and Pex20 can independently dock at the peroxisome membrane, but Pex20 is needed for the translocation of PTS2 cargo into the matrix [17]. Interestingly, the PTS2 pathway coreceptor in mammals, Pex5L, is also needed to translocate the Pex7/PTS2 cargo complex into peroxisomes [35].

The extended shuttle has been well documented for Pex20 from *P. pastoris* [17]. About two-thirds of Pex20-GFP is cytosolic in wild-type cells and the remaining one-third is peroxisomal and protease-protected, suggesting that it is in the matrix. In the absence of Pex14, a key constituent of the docking subcomplex, none of the Pex20-GFP associates with peroxisomes and all of it is cytosolic. Therefore, the docking subcomplex is necessary to translocate Pex20 into peroxiosomes. In *P. pastoris* cells lacking components of the RING subcomplex, all the Pex20-GFP is peroxisome-associated, suggesting that this subcomplex may aid the export of Pex20 from the peroxisome matrix. Finally, in the absence of any component of the receptor recycling machinery, both Pex5 and Pex20 cannot recycle from the peroxisomes to the cytosol. Under these conditions, one might expect them to accumulate on the peroxisome membrane just prior to relocation to the cytosol, but instead both Pex5 and Pex20 are degraded by a ubiquitin-proteaseome-dependent machinery responsible for a process we have dubbed RADAR [17].

The extended shuttle exhibited by the PTS1 and PTS2 receptors provides a satisfying explanation for the varying subcellular locations reported in the early studies on these proteins. The shuttle would still function as long as there is a dynamic exchange of receptors from the peroxisomes to the cytosol, irrespective of whether the predominant pool is in one or the other compartment.

The details of the receptor recycling process and the mechanism by which this is achieved are described elsewhere in this volume and will not be reiterated here. However, it is worth considering briefly how RADAR is activated and implemented.

B. The Peroxisomal RADAR Pathway

In wild-type cells there appears to be little or no turnover of Pex5 or Pex20 as long as receptor recycling is active and functional [17, 36, 37]. It is possible that receptor recycling precedes RADAR, or that the latter machinery is inactive under these conditions. Upon a block in receptor recycling (and this is true of both Pex5 and Pex20), RADAR kicks in, and the receptors that fail to recycle from the peroxisome membrane are targeted for polyubiquitylation by UBCs other than Pex4 (most likely Ubc1, Ubc4, or Ubc5 in *S. cerevisiae*)

and degradation by the proteasome [17, 36, 37]. This RADAR pathway requires specific lysines, near the N-terminus of Pex5 and Pex20, which are the targets for polyubiquitylation [17, 38]. In *P. pastoris*, K22 in Pex5 and K19 in Pex20 are necessary for RADAR [17]. Mutations of one or other of these lysines abolish RADAR for that protein [17, 38]. Interestingly, the Pex18 protein from *S. cerevisiae* is constitutively degraded in a ubiquitin- and proteasome-dependent manner [39], which is characteristic of coreceptor engagement by the RADAR pathway [17, 36, 37]. We have found, in *P. pastoris*, that Pex20 mutants that are recycled to the cytosol inefficiently behave in exactly this manner, that is RADAR now clears the peroxisome surface of cargo-free receptors (Leon and Subramani, unpublished data). Notably, these PpPex20 mutants and endogenous ScPex18 are degraded by RADAR only if the import cycle is functional [39].

Mutation of these lysines (Pex5K22R or Pex20K19R) in *P. pastoris*, or of the corresponding lysine in *H. polymorpha* Pex5, does not affect peroxisome biogenesis, consistent with the lack of a significant role for RADAR under conditions when receptor recycling is functional [3, 38]. However, in the absence of receptor recycling (due to mutations in the receptors or in the receptor-recycling machinery), these proteins accumulate at the peroxisome membrane in their polyubiuquitylated forms, providing formal evidence for the requirement of Pex4, Pex22, Pex1, and Pex6 for receptor recycling from peroxisomes to the cytosol [3]. Thus, the effects of the RADAR pathway become apparent only when receptor recycling is compromised. This provides a clue to the physiological function of the RADAR pathway, which might be to clear, from the peroxisome membrane, cargo-free receptors that cannot be recycled by other mechanisms.

C. PTS Receptor-Mediated Steps in the Matrix Protein Import Cycle

A complete understanding of the receptor shuttling pathway and mechanism requires an appreciation of the following steps (Figure 20.1):

1. *Cargo binding*: For the PTS1 pathway, most cargoes are bound by Pex5, via interactions of the TPR motifs on Pex5 with the C-terminal PTS1 peptide on the cargo [5, 40, 41]. In a few special cases, proteins such as *S. cerevisiae* acyl-CoA oxidase are imported into peroxisomes in a Pex5-dependent manner but this protein does not have a functional PTS1 [42]. Instead, the cargo protein interacts with a segment of Pex5 that is upstream of the canonical cargo-recognition site (i.e., the TPR repeats [42]).

Most PTS2 cargoes are recognized by Pex7 [12, 14], but may need proteins of the Pex20 family to stabilize this interaction, as reported for Pex18

of *S. cerevisiae* [19]. However, some cargoes, such as PpPex8 [43] and *H. polymorpha* amine oxidase [44], interact directly with Pex20. Therefore, the Pex20 family of proteins may be viewed as coreceptors for the PTS2 pathway. In plants and mammals, which do not have a Pex20-like protein, the Pex5L isoform serves as the coreceptor for the PTS2 pathway [15, 21]. Although most PTS2 proteins are recognized by their receptors or coreceptors interacting directly with the PTS2 sequence on the cargo, there are occasional exceptions to this rule, as noted above for the PTS1 cargoes. For example, *Y. lipolytica* thiolase interacts with Pex20 through a region outside the PTS2 [20].

2. *Receptor docking at the peroxisomal membrane*: Complexes formed between cargo and receptors/coreceptors interact with constituents of the docking subcomplex on the peroxisommal membrane [2]. Yeast Pex5, Pex7, and Pex20 are reported to interact independently with Pex13 and Pex14 of the docking subcomplex [3]. Pex5 and Pex20 also interact with Pex8 of this subcomplex. Although Pex17 is also a part of the docking subcomplex, none of the receptors or coreceptors appear to interact with this protein directly.

3. *Receptor/cargo translocation across the peroxisomal membrane*: It is quite likely that Pex14 and perhaps the whole docking subcomplex is necessary for the translocation of receptor/cargo complexes into the peroxisome matrix. A fraction of Pex5, Pex7, and Pex20 in yeasts is peroxisome-associated and protease-resistant, but in the absence of Pex14, Pex5 is not peroxisome-associated [43], Pex7 exhibits reduced binding to peroxisomes [45], and Pex20 is exclusively cytosolic [17]. The cotranslocation of receptor/cargo complexes is not surprising in view of the ability of peroxisomes to import folded and/or oligomeric proteins [3].

4. *Cargo release in the peroxisome matrix*: How this is achieved is not exactly clear. The environment in either the peroxisome matrix or specific proteins, such as Pex8, may be necessary, but it should be noted that the only intraperoxisomal peroxin, Pex8, is found only in yeasts, leaving open the question of how cargo release might occur in plants and mammals.

5. *Receptor export to the peroxisome membrane*: As predicted by Dammai and Subramani [27], both *cis*-acting sequences and *trans*-acting proteins are necessary for the export of receptors/coreceptors (presumably after cargo release) from the peroxisome matrix to the peroxisome membrane, from where they have to be recycled to the cytosol. The first 17 amino acids of human PEX5 are necessary for this step [46]. Similarly, the first 19 amino acids of Pex20 are required for its export [17]. Pex5 and Pex20 proteins share sequence similarities at their N-termini and these two proteins also exhibit similar dynamics and behavior during the matrix protein import cycle [3]. Hence, their mechanisms of export may be based on similar

principles. The nature of the sequences in Pex7 necessary for its export are not clear, but fusions to its C-terminus affect export efficiency [29].

Among the *trans*-acting proteins, components of the RING subcomplex, Pex2, Pex10, and Pex12, are necessary for the export of Pex20 [17]. In these mutants, Pex20 is peroxisome-associated but inaccessible to the RADAR machinery, suggesting that it is inside peroxisomes. In mammalian systems, the stage 2 state of Pex5 described earlier [47] may correspond to an intermediate that has been exported from the peroxisome matrix to the membrane, where only about 2 kDa of its N-terminus is exposed to the cytosol where it is clipped by protease. This model would be consistent with a role for the N-terminus in two distinct events—receptor export and recycling.

6. *Recycling of cargo-free receptors to the cytosol*: This probably requires monoubiquitylation of the N-termini of the Pex5 and Pex20 by Pex4 and the action of the two AAA ATPases, Pex1 and Pex6 [3, 25, 48]. The details of this process are reviewed separately in this volume.

7. *Clearance of the receptor from the peroxisome membrane when recycling is affected*: This process involves the RADAR pathway and has been described above.

D. Energetics of Receptor Recycling and Cargo Import

Peroxisomal protein import is unusual in that the entry of both receptors and cargo is ATP independent [24, 25, 49]. However, the export of Pex5 from peroxisomes to the cytosol is ATP dependent [24, 25, 49]. Part of this requirement is that the ATP-dependent UBC, Pex4, probably monoubiquitylates the receptors, as a prelude to receptor export and/or recycling [3, 25, 48]. In addition, the ATPases, Pex1 and Pex6, act in a complex to hydrolyze ATP and facilitate the relocation of the receptors from the peroxisome to the cytosol [24, 25]. Finally, as described below, Hsp70, which also hydrolyzes ATP, is necessary for matrix protein import [50, 51]. It is still unclear exactly how many ATPs are consumed for the import of each molecule of cargo.

The receptor dynamics and the sites of the energy-requiring steps in peroxisomal matrix protein import are reminiscent of nuclear protein import, where cargo enters the nucleus in association with the NLS receptor, importin, but no GTP hydrolysis is required for this step per se [52]. The energy-requiring step in nuclear import is actually in the cytosol, where GTP hydrolysis on Ran-GTP releases the import receptor for another round of cargo binding and import. In peroxisomal protein import, ATP hydrolysis in the cytosol is also necessary for replenishing receptors in the cytosol for another round of cargo binding and import [24, 25, 49].

III. Role of Hsp70 Family of Proteins in Peroxisomal Matrix Protein Import

The first evidence for the role of chaperones in peroxisomal matrix protein import came from a microinjection-based assay for the import of proteins into peroxisomes of mammalian cells [51]. This work showed an involvement of the constitutive (Hsp73), but not the inducible, heat-shock proteins (Hsps) of the 70-kDa family. Proteins of this family were associated with proteins being imported into the peroxisome matrix. Antibodies against the Hsp70 proteins inhibited peroxisomal import and the inhibition was reversed by the addition of exogenous Hsp70. This class of proteins was found associated with purified rat liver peroxisomes, and more peroxisome-associated Hsp70s were found during peroxisome proliferation. Interestingly, protease protection assays suggested that the association of Hsp73 terminated at the peroxisome membrane because Hsp73 remained protease-sensitive. However, when an unfolded protein, such as reduced, alkylated, and biotinylated human serum albumin conjugated to a peptide ending in the PTS1, SKL (bHSA-SKL), was microinjected into mammalian cells, Hsp70 associated with the cargo and was found inside the peroxisomes [53]. The rate of peroxisomal import of folded and unfolded bHSA-SKL was essentially the same [53]. The ability of chaperones to enter peroxisomes in association with unfolded proteins may circumvent the absence of chaperones in mammalian peroxisomes. The roles of intra- and extraperoxisomal Hsp70 in the import cycle may be quite distinct.

Proteins of the Hsp70 family function as ATPases, like the DnaK protein of *Escherichia coli* [54]. These proteins exist is two conformational states, one bound to ADP and with a higher affinity for protein/peptide binding, and the other bound to ATP and possessing a lower affinity for protein/peptide binding. The ATP hydrolysis rate, and hence the conversion of Hsp70 to the ADP-bound form, for this family of proteins is stimulated by members of the DnaJ, or Hsp40, family [55]. Thus, it was anticipated that members of this Hsp40 family would also be required for peroxisomal matrix protein import. In mammalian cells, this is indeed true as judged by the inhibition of import in a permeabilized mammalian cell system by antibodies specific to Hsp40 or Hsp70 [41]. Although the requirement of members of the Hsp70/Hsp40 family for peroxisomal matrix protein import was first demonstrated for PTS1-containing proteins [51], subsequent work in permeabilized mammalian cells revealed that this was also true for PTS2-containing proteins [56]. Thus, all or most peroxisomal matrix proteins need these chaperones for their import. Lending further support to this idea is the finding that among the mammalian organellar proteins that bound to *E. coli* Hsp70 were peroxisomal matrix proteins (the multifunctional enzyme and an isoform of 2,4-dienoyl-CoA reductase) [57].

The role of the Hsp70 family of proteins is not well studied in yeast systems that have been so instrumental in advancing our knowledge of the mechanism of peroxisome biogenesis. A member of the DnaJ family, Djp1p, has been reported to be involved specifically in the import of peroxisomal matrix proteins [58]. Cells lacking this protein were impaired only in peroxisomes and peroxisomal matrix protein import was affected to various extents. However, nuclear, endoplasmic reticulum (ER) and mitochondrial import were not affected.

Many members of the Hsp70 family also act in concert with nucleotide exchange factors that replace ADP with ATP (e.g., the BAG family of proteins in eukaryotes) [54]. No specific exchange factor has been identified to play a role in peroxisomal matrix protein import. However, there is indirect, suggestive evidence for the involvement of a plant nucleotide exchange factor in the targeting of a peroxisomal membrane protein, cottonseed ascorbate peroxidase (APX), to the ER, from where it is proposed to be sorted to peroxisomes [59].

In plants, there are several reports of the existence of Hsp70 family proteins on or in peroxisomes, as well as of their requirement for peroxisome biogenesis. An Hsp70 homologue from *Citrullus vulgaris* was shown to be targeted to both chloroplasts and peroxisomes by initiation of protein translation at two different methionines [60]. The peroxisomal variant has a PTS2 sequence. A DnaJ (Hsp40) homologue from *Cucumis sativus* was found to be attached to the glyoxysomal membrane, in association with a cytosolic Hsp70 [61]. Plant peroxisomal protein import *in vitro* is enhanced by the presence of chaperones [50]. Peroxisomes isolated from heat-shocked pumpkin seedlings showed more import than those from untreated seedlings. Additionally, antibodies to wheat germ Hsp70 (and *E. coli* Hsp90) inhibited peroxisomal import of isocitrate lyase. Finally, immunoprecipitates of Hsp70 contained peroxisomal matrix proteins.

The posttranslational import of a plant peroxisomal membrane protein, APX, into purified ER membranes (from where they are proposed to sort to the peroxisomes) *in vitro*, was also impaired by the immunodepletion of Hsp70, AtJ2 (a DnaJ homologue), and AtE1 (an *E. coli* GrpE homologue) [59].

Small heat-shock proteins (sHsps; 16–42 kDa) with PTS1- and PTS2-targeting sequences have been described in plants [62]. Although the function of these proteins is unclear in plants, in other systems they prevent protein aggregation.

In other organelles that transport unfolded proteins across their membranes (ER, mitochondria, and chloroplasts), the requirement for Hsp70/40 proteins is not surprising because these chaperones help to maintain the newly synthesized proteins in the unfolded state prior to translocation

across the membranes. However, the peroxisomal translocon is unusual in that folded, oligomeric and cofactor-bound proteins can be translocated across the peroxisomal membrane [3]. In view of this, the exact role of Hsp70 remained an enigma but three models have been proposed [63].

In the first model, the chaperones might stabilize the exposed C-terminal PTS1 sequence during thermal fluctuations that unfold the C-terminal end of the protein, without unfolding the rest of the protein. This would predict that the presence of chaperones might aid the interactions between the PTS receptor and its cargo. The second model suggests that Hsp70 proteins might help to facilitate assembly of the cargo/receptor complexes with docking proteins on the surface of peroxisomes. The final model is one where Hsp70/Hsp40 act in a manner analogous to clathrin-uncoating ATPase, to disassemble protein complexes on the peroxisome membrane. In the light of current knowledge regarding the extended shuttling and recycling of PTS receptors, an obvious possibility is a late requirement for Hsp70 in the receptor-release step at the peroxisome membrane.

The binding between the purified PTS1 receptor domain that binds cargo and a PTS1 peptide was studied by fluorescence anisotropy [64]. Specific binding of the receptor to the PTS1 peptide was observed, but no effect was seen on addition of Hsp70, Hsp70 and ATP, or Hsp70 and ADP. However, in these experiments, no Hsp40 was added. In a conflicting report, it was shown that mammalian Hsp70 interacts with the cargo-binding (TPR) domain of PEX5, and that Hsp70 and ATP synergistically enhance the binding of PEX5 to the PTS1 of acyl-CoA oxidase, a peroxisomal matrix protein [65]. However, the energy dependence of cargo binding is not consistent with other reports that cargo binding and import into peroxisomes do not require ATP [24].

Therefore, at present it is still unclear exactly how and when Hsp70/Hsp40 proteins act during peroxisomal matrix protein import.

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