The (life) cycle of Pex5p:

The role of PTS1 receptor Pex5p in the import of proteins into the peroxisome

Matthijs Schokker
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Ida van der Klei

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Abstract

The import of proteins into peroxisomes is a peculiar process in which folded and even oligomeric proteins can be translocated across the peroxisomal membrane. Here we provide a review of the role of the receptor of peroxisome targeting signal 1, Pex5p. Pex5p plays an important role in the import of proteins into peroxisomes, as it is involved in the binding of the targeted proteins, it recruits the cargo the peroxisomal membrane, it binds to the import machinery or can even be part of it. The mechanics of the translocation are still largely unknown, but the major hypothesis is the formation of transient pores with Pex5p as a constituent of the pore complex or driver of the pore complex formation. Pex5p is recycled after translocation by monoubiquitination and subsequent removal from the membrane by the Pex1p and Pex6p, comprising the only energy dependency in the import process. Concluding, we propose an extension to transient pore model.

Abbreviations

ER: endoplasmatic reticulum, one of cell organelles.
PTS 1 or 2: peroxisomal targeting signal I or II, amino acid sequences in proteins that are targeted to the peroxisomal matrix.
SKL motif: Serine-Lysine-Leucine motif, the canonical PTS 1 sequence.
TPR: tetratricopeptide repeat, domain of Pex5p involved in PTS 1 recognition.
UBPex5p: monoubiquitinated Pex5p.

Introduction

Organelles, compartments for specific purposes, play a major role in the life of eukaryots. Whereas prokaryots only possess basic temporal localization of reactions, in eukaryots many biochemical reactions are placed inside specific organelles. This allows for additional regulatory processes. For instance, the nucleus, with inside the DNA and transcription from DNA to mRNA, is separated from ribosomes, which are inside the cytosol, allowing for distinct transcriptional and translational regulation [Alberts *et al.*, 2002].

One type of organelles is the peroxisome. Peroxisomes consist of a single bilayer membrane, which contains a relatively low amount of proteins [Van Der Klei and Veenhuis 2002]. They can be formed *de novo* from the endoplasmatic reticulum (ER), through the clustering of certain peroxisomal membrane proteins (PMPs) in foci and subsequent budding off from the ER [Kim *et al.*, 2006; Hoepfner *et al.*, 2005; Tabak *et al.*, 2003]. Next to the *de novo* formation, peroxisomes can also proliferate by fission and can be inherited by daughter cells, for example in yeast buds [Schrader *et al.*, 2012; Opaliński *et al.*, 2011; Fagarasanu *et al.*, 2006].

Which enzymatic reactions are performed in a peroxisome depends on the protein content of the peroxisome. By definition H_2O_2-producing reactions take place, coupled to the enzyme catalase, which degrades H_2O_2 to water and molecular oxygen. Generally, also the β-oxidation of fatty acids takes place in peroxisomes [Titorenko and Rachubinski, 2001]. Depending on tissue and organism more specific functions, like parts of the glycolysis, the synthesis of plasmalogens, or the metabolism of methanol can be localized in the peroxisome [Moyersoen *et al.*, 2004; Brites *et al.*, 2004; van der Klei *et al.*, 2006]. Furthermore, several of these more specialized functions give rise to a range of subtypes of peroxisomes, like Woronin bodies, which close septum pores in fungi [Lanyon-Hoog *et al.*, 2012; Jedd and Chua, 2000].

As described above, the protein content of peroxisomes is important for their function. The protein concentration in the peroxisome can be so high that these peroxisomal matrix proteins can be seen as a crystalloid structures on electron microscopy images [Van Den Bosch *et al.*, 1992]. Another striking feature is the import of folded and even oligomeric proteins into the peroxisome [Glover *et al.*, 1994; McNew and Goodman 1994; Titorenko *et al.*, 2002]. One study even reports entry of up to 9nm gold particles [Walton *et al.*, 1995]. In contrast, proteins are imported unfolded or with hairpin structures in most other organelles. Another exception is the nucleus in which folded proteins can be imported via the nuclear pore complexes. However, in peroxisomes no such stable pores have been observed [Erdmann and Schliebs, 2005; Lanyon-Hogg *et al.*, 2012]. In this sense the peroxisome protein import pathway resembles the twin arginine translocase protein export pathway that is found in bacteria, which also exports folded proteins over a single bilayered membrane, without disrupting the gradient of small molecules and ions [Palmer and Berks, 2012].

This begs the question, how are perixomal matrix proteins imported? Generally proteins are targeted to the peroxisomal matrix by one of two peroxisomal targeting signals (PTS), type I or type II [Rucktaschel *et al.*, 2011]. The extreme C-terminal tripeptide of the PTS1, which has the consensus sequence (S/A/C)-(K/R/H)-(L/M) (called SKL motif for rest of article) [Lametschwandtner *et al.*, 1998]. Because in some cases additional residues are required for binding of a PTS1 protein to the receptor, the PTS1 can better be defined as 'a C-terminal dodecamer' [Brocard *et al.*, 2006; Rucktaschel *et al.*, 2011]. The PTS 2 sequence has the consensus sequence (R/K)-(L/V/I)-$(X)_5$-(H/Q)-(L/A), in which X depicts any amino acid, and the sequence is near the N-terminus [Rachubinski and Subramani, 1995]. In contrast to the PTS1, the PTS2 signal is often cleaved off after translocation into the peroxisome [Osumi *et al.*, 1991; Swinkels *et al.*, 1991]. The two different signals are recognized by two different receptors, Pex5p for PTS1 and Pex7p for PTS2 [Brocard *et al.*, 1994; Terlecky *et al.*, 1995; Rehling *et al.*, 1996]. Whereas Pex5p can solely escort the cargo protein to the peroxisome, Pex7p needs a co-receptor. The identity of this co-receptor is species specific, *i.e.* a long isoform of PEX5, PEX5L, in mammals and Pex20p in *Hansenula polymorpha* [Otzen *et al.*, 2005; Otera *et al.*, 2000; Matsumura *et*

al., 2000]. These receptors cycle between cytosolic localization, bound to the peroxisomal membrane and back to the cytosol [Lanyon-Hogg *et al.*, 2012]. A schematic representation of these cycles can be found in figure 1. The cycle of Pex5p will be the subject of this essay.

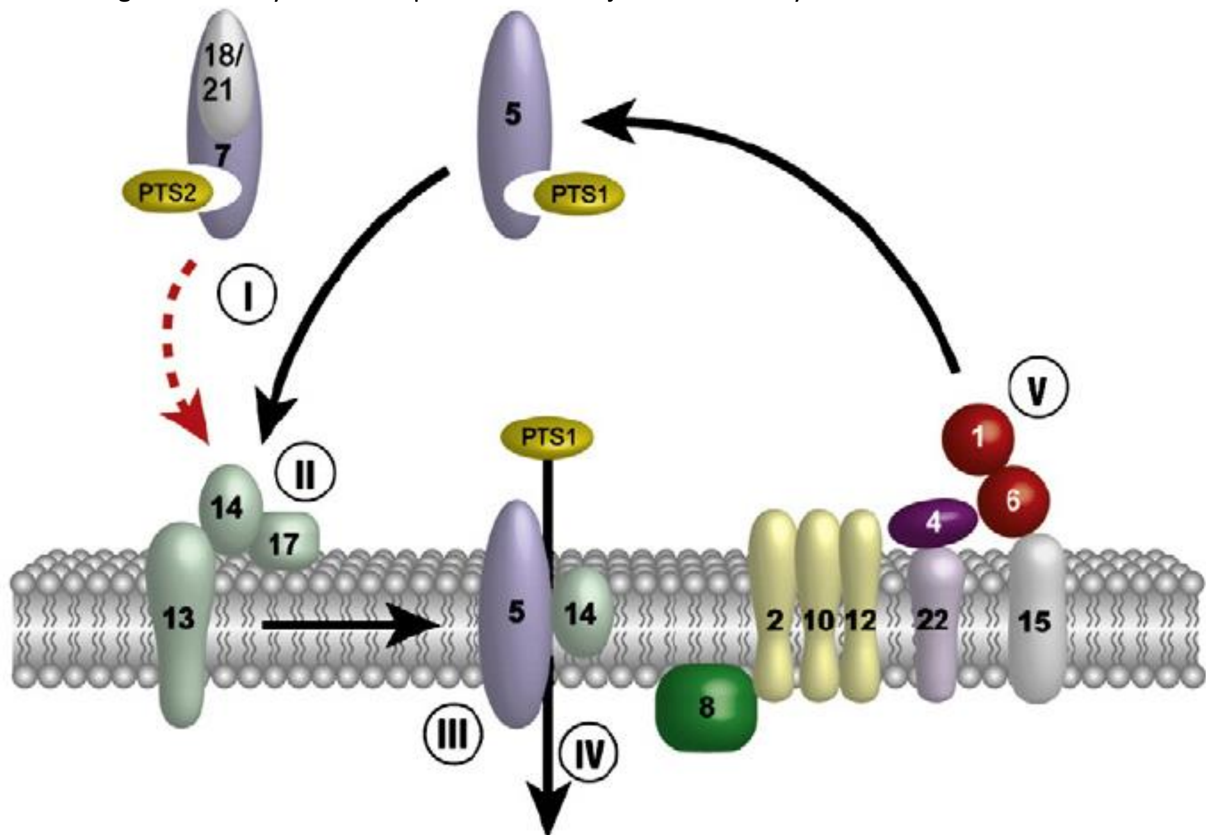

Figure 1: Schematic representation of the import of peroxisomal matrix proteins in *Saccharomyces cerevisiae*. The numbers inside the symbols indicate which peroxin it is. Shown for the PTS2 recognition are the *S. cerevisiae* co-receptors Pex18p/21p. The roman numerals indicate the distinct steps in the import process. (I) PTS recognition; (II) docking of receptor-cargo complex to the docking complex; (III) transport of cargo across the membrane; (IV) release of cargo into the peroxisome; (V) receptor ubiquitination and recycling. Image taken from Rucktaschel *et al.*, 2011.

The (life)cycle of Pex5p

PTS 1 recognition

Newly synthesized PTS 1 carrying proteins are recognized by Pex5p. In this recognition seven tetratricopeptide repeat (TPR) domains of Pex5p are important [Dodt *et al.*, 1995; Gatto *et al.*, 2000]. TPRs are 34 amino acid domains that are often repeated. They fold into two α-helices, connected via a loop. TPR domains 1-3 and 5-7 are primarily involved in the binding of the SKL motif, with TPR 4 functioning as a hinge, together forming a ring-like structure with a groove in the centre where the PTS 1 peptide is bound. This was indicated by the crystal structure of Pex5p, bound to a pentapeptide [Gatto *et al.*, 2000]. A detailed account of the interactions of Pex5p residues and the SKL motif can be found in the review of Lanyon-Hogg from 2012, and the references therein.

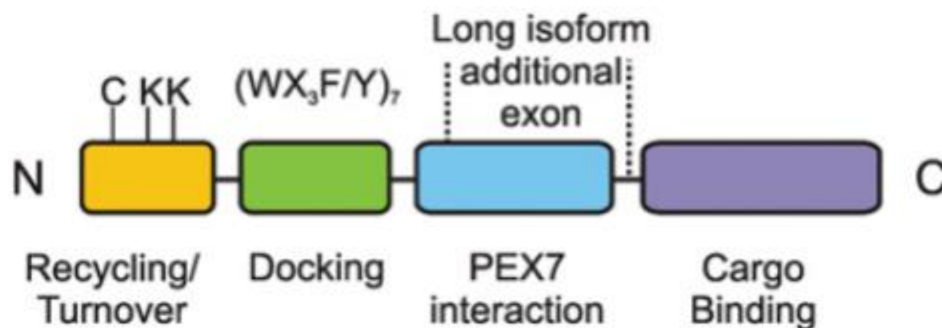

Figure 2: Schematic representation of human PEX5L. Several different domains, with each its own function, are depicted. The cysteine and two lysines are targets for mono- and polyubiquitination, respectively. The W-X_2-(F/Y) repeats are important in the binding of PEX14. The PEX7 interacting domain is specific for mammalian PEX5L. C-terminal part contains the TPR domains. Image taken from Lanyon-Hogg *et al.*, 2012.

Binding to docking complex

The next step in the transport of proteins to the peroxisome is the binding of Pex5p to the peroxisomal membrane, which is facilitated by the membrane docking complex, which consists of peroxisomal membrane proteins (PMP) Pex13p, Pex14p (and Pex17p in yeast) [Subramani *et al.*, 2000]. Furthermore, under influence of Pex8p, the docking complex interacts with Pex2p, Pex10p and Pex12p [Agne *et al.*, 2003].

Of Pex13p both the C and N-terminus are located on the outside of the peroxisomal membrane [Girzalsky *et al.*, 1999]. Pex5p interacts with the Scr homology 3 (SH3) domain that is located in the C-terminus of Pex13p [Erdmann and Blobel, 1996; Elgersma *et al.*, 1996; Gould *et al.*, 1996]. SH3 domains are ~60 amino acid long domains that can form an independent module in many proteins and are found in different types of proteins, like those involved in signal transductions [Musacchio *et al.*, 1994]. The SH3 domain of Pex13p is also used in the interactions with Pex14p [Subramani *et al.*, 2000].

Pex14p, the other essential protein in the docking complex, is a transmembrane protein of which the N-terminus is protected against chemical and proteolytic cleavage. It is able to form homodimers and oligomers [Will *et al.*, 1999; Oliveira *et al.*, 2002]. This N-terminus is also the place of interaction with the W-X_2-(F/Y) domains in Pex5p. The aromatic residues of the W-X_2-(F/Y) domain of Pex5p are caught in hydrophobic pockets of the first two helices in Pex14p [Lanyon-Hogg *et al.*, 2012, and references therein].

Since the docking complex is able to bind both Pex5p and Pex7p, this is where the PTS 1 and PTS 2 paths converge [Rayapuram and Subramani 2006]. Strikingly, PTS 1 transportation seems only partly dependent on the docking complex, since deletion of the interacting domains of Pex5p does

not abolish all PTS 1 import. This seems to indicate that Pex5p can enter the membrane unassisted [Kerssen *et al.*, 2006]. Furthermore, the insertion of Pex5p is dependent on the presence of a cargo protein [Gouveia *et al.*, 2003].

Cargo release and translocation across the membrane

As one would expect, the entire cycle of Pex5p, as depicted in figure 1, needs energy, in the form of ATP, to be active. Strikingly, this ATP dependency is not due to the import of Pex5p-cargo protein complex into the peroxisome, but to the export of Pex5p [Oliveira *et al.*, 2003]. It is still unknown if any additional driving force is needed for the import of proteins, or how the precise pore formation and translocation is achieved.

In 2005 Erdmann and Schliebs proposed the existence of a transient pore in the peroxisomal membrane through which protein import could concur. In this model the import receptor would also function as an integral part of the translocation machinery, in resemblance to pore forming toxins [Erdmann and Schliebs 2005].

To test the hypothesis that import receptors themselves contributed to translocation activity, the lab of Erdmann reported on dynamics of reconstituted pores [Meinecke *et al.*, 2010]. In this article they isolated tomato etch virus tagged Pex5p by affinity chromatography and subsequently defined several stages between monomeric Pex5p and oligomeric complexes using size exclusion chromatography. Three distinct complexes were named complex 1-3. Complex 1 constituted of a PTS 1 containing protein and Pex5p. Complex 3 was concluded to be the assembled importomer. Complex 2 consisted of Pex5p and the Pex14p/Pex17p heterodimer Pex13p in a part of the complexes, but lacking cargo-protein and the export machinery. To assert the potential of these complexes for pore formation, the complexes were reconstituted into liposomes and measured in a planar lipid bilayer setting for ion-conductance, from which the diameter of the pore can be calculated. The lipid bilayer technique is a relatively new experiment to determine the ability of proteins or protein complexes to form pores in a bilayer membrane. Two chambers are separated by a wall with a hole in it that can be closed by a membrane, sealing the chambers and inhibiting diffusion from one chamber to the other. When pores are formed by the complex a current will form that can be measured and used to calculate the size of the pore [Harsman *et al.*, 2011].

Pores formed by complexes 2 and 3 were stable when Pex8p and Pex18p and Pex21p were deleted, of which Pex8p links the docking complex to the export complex and Pex18p and Pex21p are the co-receptors in yeast for the PTS 2 import. However, only upon incubation of the complexes with isolated cytosolic Pex5p-cargo protein it was possible to achieve larger pores, with leading to conductances of up to 1150pS, corresponding to a functional size of 3.8nm (see figure 3). As a last step they were able to create pores with a conductance of up to 2800 pS (9.25nm) when Pex5p carrying a large oligomeric protein, Fox1p, was used for incubation. This size would allow for the transport of large oligomeric protein complexes and even the aforementioned gold particles.

A possible problem with the article arises because of the calculation from conductance to pore size. For this calculation it is important to know the resistivity of the pore, which is dependent on the structure. As the structure of the pore is unknown, the calculation of the pore size is a rough estimation [Harsman *et al.*, 2011]. Since no calculation or rational is given by Meinecke *et al.* in their article, the calculated number of 9,25nm has the appearance of being manipulated to be close to the 9nm gold particles. Although there is no strong evidence for this accusation, it should be taken in account as a possibility, weakening the strong statements made in their article. It is clear however, that the pore conductance increases upon incubation of the pore complex with Pex5p-cargo protein complexes. Additionally, larger Pex5p-protein complexes seem to allow for larger pores.

Although pore formation by the protein import complex was observed, proper pore formation was only observed after deletion of Pex8p, which is thought to link the importomer to the export complex [Agne *et al.*, 2003]. Furthermore, the observed pore formation did not lead to the translocation of the cargo over the membrane, calling its relevance into question [Meinecke *et al.*,

2010]. At least one factor would then be essential for the actual translocation through the formed pore, either an unknown factor in the cytosol or peroxisome itself or the export complex. Since it is possible to add factors to specifically one side (*trans* or *cis*) of the membrane, it would be interesting to see if the addition of Pex8p on the *trans* side of the membrane could restore protein translocation. Additionally, when Pex8p is not sufficient, Pex26p could be reconstituted in the membrane and Pex1p and Pex6p (with ATP) could be added to the *cis* side of the membrane. This would allow for testing the hypothesis that protein import is dependent on the export of Pex5p.

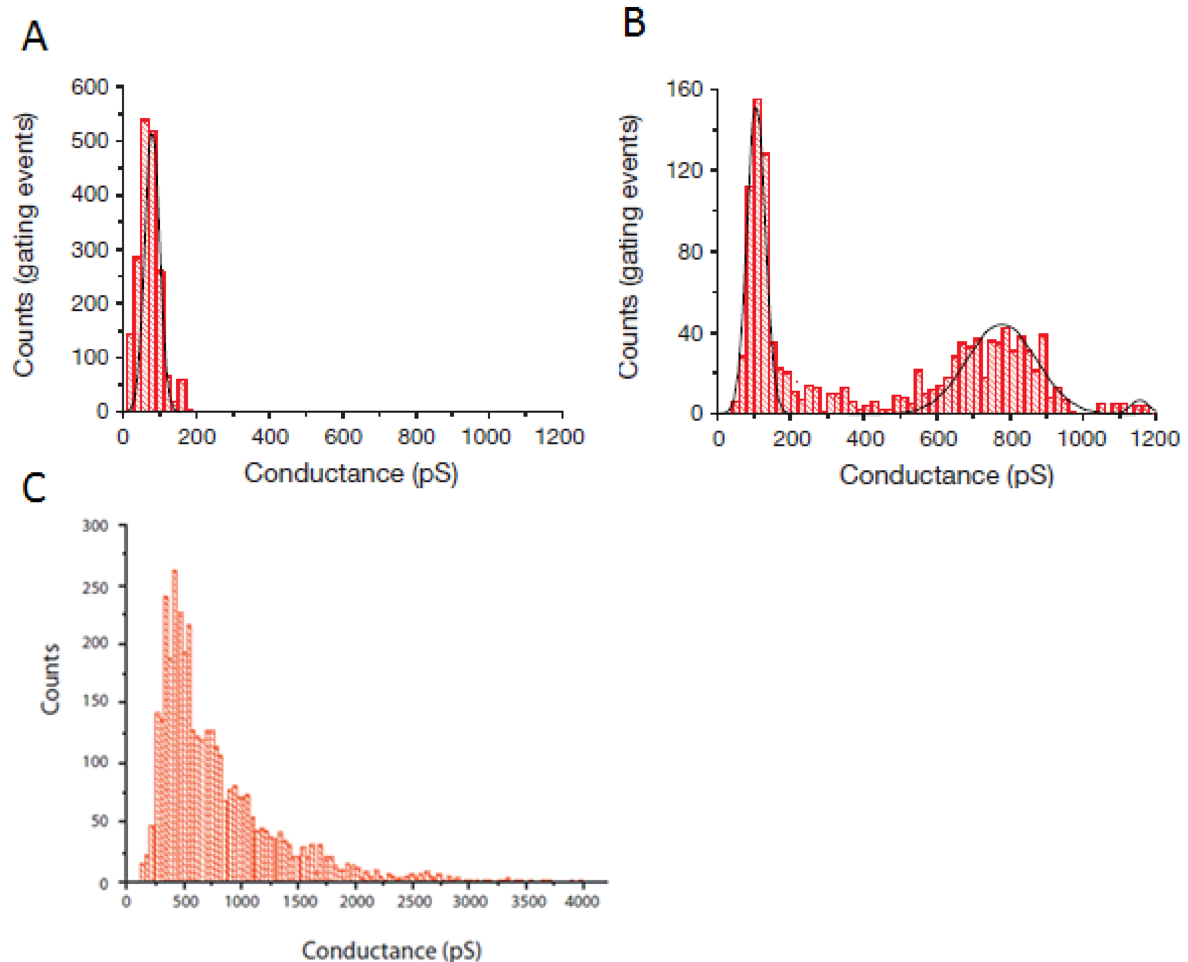

Figure 3: Conductance states histograms of non-activated (**A**) and activated (**B** and **C**) import complexes. Non-activated complexes are isolated import complexes, containing Pex5p, Pex14p/, Pex17p and to lesser extend Pex13p, were reconstituted into liposomes and subsequently used for lipid bilayer measurements, without further treatment. Activated complexes were incubated with Pex5p from the cytosol before lipid bilayer measurements. For activation either the entire Pex5p population from the cytosol was used (**B**) or Pex5p bound to Fox1p (**C**). Images taken from Meinecke *et al.*, 2010.

One other question that remains is which proteins are essential for translocation of matrix proteins over the membrane. Taking in account the dynamic and transient nature of the proposed pore and the apparent influence of the size of the PTS 1 bound protein on the size of the pore, it is tempting to speculate that the essential components for pore formation depend on the need. So depending on the size and whether or not they are imported as multimers, either Pex13 or Pex14 is essential for the insertion of the cargo into the peroxisome. Supporting this hypothesis are two studies in which human catalase where either Pex13 or Pex14 are essential, for tetrameric and monomeric catalase respectively [Otera and Fujiki, 2012; Freitas *et al.*, 2011]. However, some

indications exist that Pex13p is more important at the initial docking of Pex5p, than at its transport Meinecke *et al.*, 2010). A possible experiment to test for the differential roles of Pex13p and Pex14p would be a lipid bilayer experiment using import complexes from *PEX13* and *PEX14* deletion strains.

In their article, Meinecke *et al.*, speculate about the involvement of Pex5p itself in pore formation. As Pex14p from a *PEX5* deletion strain was not able to form pores they concluded that Pex5p itself plays a pivotal role in the architecture of the pore. Supporting this hypothesis, it was found that Pex5p is able to enter membranes on itself [Kerssen *et al.*, 2006]. Furthermore, a subpopulation of Pex5 acted as an integral membrane protein in rat liver cells [Gouveia *et al.*, 2000]. We could speculate on the role of the natively disordered domains of Pex5p in the binding of membranes, but no evidence suggests that it is involvement in binding of membranes [Stanley and Wilmanns, 2006]. However, these data do not provide conclusive evidence for a role of Pex5p in the architecture of the pore.

Another possibility is that interaction of Pex5p could induce conformational changes in the importomer, leading to pore formation; *i.e.* oligomerization of pore forming peroxins, in analogy to the twin arginine translocase system [Palmer and Berks, 2012]. This possible explanation is supported by a study reporting that upon binding of Pex5p Pex14p undergoes conformational changes in the protozoa *Leishmania donovani* [Cyr *et al.*, 2008]. Furthermore, in a recent study Natsuyama *et al.*, found that a Pex5 disabling mutation led to a decreased stability of Pex14p, which they attributed to a decreased localization to the peroxisomal membrane [Natsuyama *et al.*, 2013]. Additional research on this topic is needed and could for instance include an experiment in which Pex5p is mutated in the natively unfolded domains so no amphipathic helices can form. This mutant can then be analyzed based on its ability to insert into membranes, form pores *in vitro* and on its ability to translocate cargo proteins *in vivo*.

Finally, the release of proteins into the peroxisomal membrane is still not well known. Several factors are known, however. Wang *et al.*, showed that Pex5p *in vitro* tetramerized in cytosolic conditions (pH 7.2), which was associated with the binding of a PTS1 peptide. The PTS1 peptide was released upon a decrease in pH (to pH 6.0) and with the binding of Pex8p [Wang *et al.*,. 2003]. The release of cargo proteins upon binding of Pex8p could be attributed to the PTS 1 and PTS 2 signals present on Pex8, which is present at the inside of the peroxisomal membrane [Platta *et al.*, 2013]. Another study reported the release of monomeric catalase upon binding of Pex5p to Pex14p [Freitas *et al.*, 2011]. The latter study suggested a model in which Pex5p upon binding to Pex14p releases its cargo in the direction of Pex14p forcing the cargo to go inside. However, since protein import is not solely dependent on Pex14p this is not likely [Kerssen *et al.*, 2006; Salomons *et al.*, 2000].

Ubiquitination and receptor recycling

As a final step in the cycle of Pex5p it needs to be released from the peroxisomal membrane. This process is ATP-dependent, as discussed above. The process of removing Pex5p from the membrane is now viewed as a two-step process, with first the monoubiquitination of Pex5p and afterwards the removal of Pex5p through two AAA ATPases [Fransisco *et al.*, 2013]. The export of proteins from peroxisomes are similar to that of the ER associated protein degradation (ERAD) in the method of protein export, although in the ERAD system proteins are always polyubiquitinated before export [Grimm *et al.*, 2012].

Ubiquitination has been known for a long time as a process of quality control. Canonically, multiple rounds of ubiquitination results in several ubiquitin molecules on the lysine residue of a protein that is targeted for degradation. Ubiquitin is a peptide which is being activated and afterwards covalently bound to the target protein, creating an isopeptide bond between the C-terminus of ubiquitin and the ε nitrogen of lysine. Since ubiquitin itself also has lysine residues that can be targeted, a string of ubiquitin molecules can be formed on a single lysine residue of the targeted protein. This polyubiquitination is in turn a signal for the proteasome to degrade the tagged

protein [Haglund and Dikic, 2012]. Three distinct enzymatic reactions are important. in ubqcuitination cascade. One type of enzyme is distinguished for each step, E1-3. E1 enzymes phosphorylate of ubiquitin to activate the peptide. E2 enzymes bind the ubiquitin bring it tothe target. E3 enzymes perform the catalytic reaction in which ubiquitin is ligated to the target protein [Hershko *et al.*, 1992].

An additional role for ubiquitination has been found in many cellular processes, in which proteins are ubiquitinated only once [Fransisco *et al.*, 2013, and references therein]. In the case of Pex5p, two types of ubiquitination can occur, monoubiquitination of a conserved N-terminal cysteine or the polyubiquitination of two N-terminal lysine reidues. The monoubiquitination of Pex5p leads to recycling of the receptor to the cytosol, since after removal UbPex5p can be passively or actively be deubiquitinated [Platta *et al.*, 2007; Carvalho *et al.*, 2007] The polyubiquitination is thought to be a quality control for Pex5p molecules, activated upon the disability of Pex5p to leave the peroxisomal membrane [Kiel *et al.*, 2005; Kragt *et al.*, 2005].

Monoubiquitination of Pex5p is thought to be performed by four proteins, Pex2p, Pex4p, Pex10p and Pex12p. Pex4p is a E2 protein in yeast that is recruited to the membrane by Pex22p. In mammals no homologue of Pex4p was discovered, but seems to be taken over by the cytosolic E2 enzymes UbcH5a-c [Grou *et al.*, 2008; Williams *et al.*, 2008]. Deletion of PEX4 resulted in heavily decreased levels of pex5p, which were returned to normal upon deletion of members of the transportation module [Collins *et al.*, 2000]. Since many E3 enzymes are RING proteins, the RING like proteins Pex2p, Pex10p and Pex12p were early on thought of as the E3 enzymes in the ubiquitination of Pex5 [Fransisco *et al.*, 2013]. They were also proven to exhibit E3 enzymatic activity towards mono- and polyubiquitination of Pex5p [Williams *et al.*, 2008; Platta *et al.*, 2009].

To test which of these proteins was responsible for the monoubiquitination of Pex5, Platta *et al.*, designed an experiment in which the RING domains of Pex2 Pex10 and Pex12 was substituted with ProtA to abolish its E3 ligase activity, without influencing the stability of the protein complex. In using different combinations of mutants, only in the complex with Pex12 could some monoubiquitination be seen, while mutation of the Pex12 RING domain abolished monoubiquitination activity. From these results they concluded that Pex12 was responsible for this modification [Platta *et al.*, 2009]. Lending some support to their hypothesis is the observation that Pex2 and Pex10 have a normal RING topology, whereas Pex12 only coordinates only one of the normally two Zn^{2+} ions, suggesting it could have a different subset in targets [Koellensperger *et al.*, 2007]. However, much E3 ligase activity was lost when only Pex12 was functional. Furthermore, substitution of a single one of the RING domains of Pex2, Pex10 and Pex12 with ProtA inhibited import of PTS 1-GFP [Platta *et al.*, 2009]. So the option still remains that multiple proteins are needed for the proper monoubiquitination of Pex5 [Fransisco *et al.*, 2013].

After ubiquitination Pex5p is recognized by two members of the exportomer, Pex1p and Pex6p. These two AAA-type ATPases recognize the ubiquitinated Pex5p and remove it from the membrane.

As many other AAA-type ATPases, Pex1 and Pex6 form a ring formed oligomer with an internal cavity [Iyer *et al.*, 2004]. This complex is hexameric in the case of Pex1 and Pex6 and has a 1:1 ratio of both proteins [Saffian *et al.*, 2012]. Complex formation is dependent on two AAA cassettes, D1 and D2 of both proteins and subsequently on the binding of ATP to the D2 cassette of Pex1p in yeast, but not its hydrolysis [Birschmann *et al.*, 2005; Tamura *et al.*, 2006]. In mammals binding of ATP to both the first and second ATPase cassettes is required for complex formation [Tamura *et al.*, 2006]. In the formed hexamer Pex6p is recognized by Pex26p (Pex15p), which is located in the peroxisomal membrane, and recruited to the peroxisome [Matsumoto *et al.*, 2003; Tamura *et al.*, 2006]. Contradictory data exists on the need to form ternary structures of Pex1p, Pex6p and Pex26p before localization to the peroxisome. Whereas some studies found that Pex6p is recognized and recruited by Pex26p independent of Pex1p [Matsumoto *et al.*, 2003; Weller *et al.*, 2005], whereas another study showed a cytosolic localization of Pex6p in the absence of Pex1p

[Tamura *et al.*, 2006]. In the latter study they speculate on whether Pex1p stabilizes Pex6p on the peroxisome or that other undetected proteins interact with Pex1p and Pex6p.

Two years ago a, Miyata *et al.*, discovered another partner of Pex1 and Pex6, that is required for the binding and translocation of Pex5p, namely AWP1 [Miyata *et al.*, 2011]. AWP1 is a cytosolic protein that was already known to interact with ubiquitinated NF-κB through one of the zinc-finger domains, which is also responsible for binding of ubiquitinated Pex5 [Miyata *et al.*, 2011 and references therein]. Both zinc finger domains are also required for binding of AWP1 to Pex6. Knockdown of AWP1 led to a sharp decrease in protein import, measured by SKL-EGFP and Alcohol Oxidase levels, and to a decreased stability of Pex5, seen as a 50% reduction of Pex5 levels [Miyata *et al.*, 2011]. As Pex1p and Pex6p do not directly bind to Pex5p for removal, AWP1 can function as a Pex5p-Ub selective adapter.

Together Pex1p and Pex6p are proposed to pull the marked receptor out of the membrane, freeing it for subsequent degradation, as is observed in other AAA-type ATPases, in the case of polyubiquitination, or receptor recycling, in the case of monoubiquitination [Fujiki *et al.*, 2012; Grimm *et al.*, 2012]. As the ATP hydrolysis in Pex1p and Pex6p is the only energy source for peroxisomal import, apart for ATP required for ubiquitination, it is tempting to speculate on a model in which the import of Pex5p and the cargo proteins is energetically driven by the export, as Schliebs *et al.*, suggested [Schliebs *et al.*, 2010]. A proposed mechanism for this export-driven import is for instance by conformational changes made to Pex5p when it is part of the pore, thereby inducing translocation or clearing the pore for translocation, or by freeing the pore of the already translocated receptor [Schliebs *et al.*, 2010]. Further research is required to validate this theory.

Conclusion

Although Pex5p is an essential protein for normally functioning peroxisomes, still a lot is unknown about how it facilitates the crossing of proteins over the peroxisomal membrane. Models have been proposed on the mechanics of this translocation, with currently the most influential being that Pex5p inserts in the membrane and interacts with Pex14p, and to lesser extend Pex13p and Pex17p, to form dynamic, transient pores that allow for the translocation of folded, even oligomeric proteins. The energy that would be needed for the translocation is solely contributed to the removal of Pex5p from the peroxisomal membrane, by Pex5p monoubiquitination and subsequent function of the AAA ATPases Pex1p and Pex6p.

We would like to propose an extended model, in which Pex13p and Pex14p have the potential to interact in the membrane. This interaction is induced by binding of Pex5p-cargo protein to these proteins, leading to oligomerization of Pex13p and Pex14p, creating a transient pore complex. Pex5p acts as a cap for this pore, inhibiting the release of molecules that are enriched in peroxisomes, like H_2O_2. Furthermore, as Pex5p interactions not solely on the *cis* side of the protein, but also on the *trans* side, it pushes or pulls itself and the cargo protein through the pore, coupled to a release of the cargo protein upon interaction with Pex8p. Upon release of cargo proteins the core complex becomes unstable and Pex5p is pulled out of the membrane by ubiquitination and subsequent AAA ATPase activity.

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