THE ROLE OF PEX5 IN PEROXISOMAL MATRIX PROTEIN IMPORT

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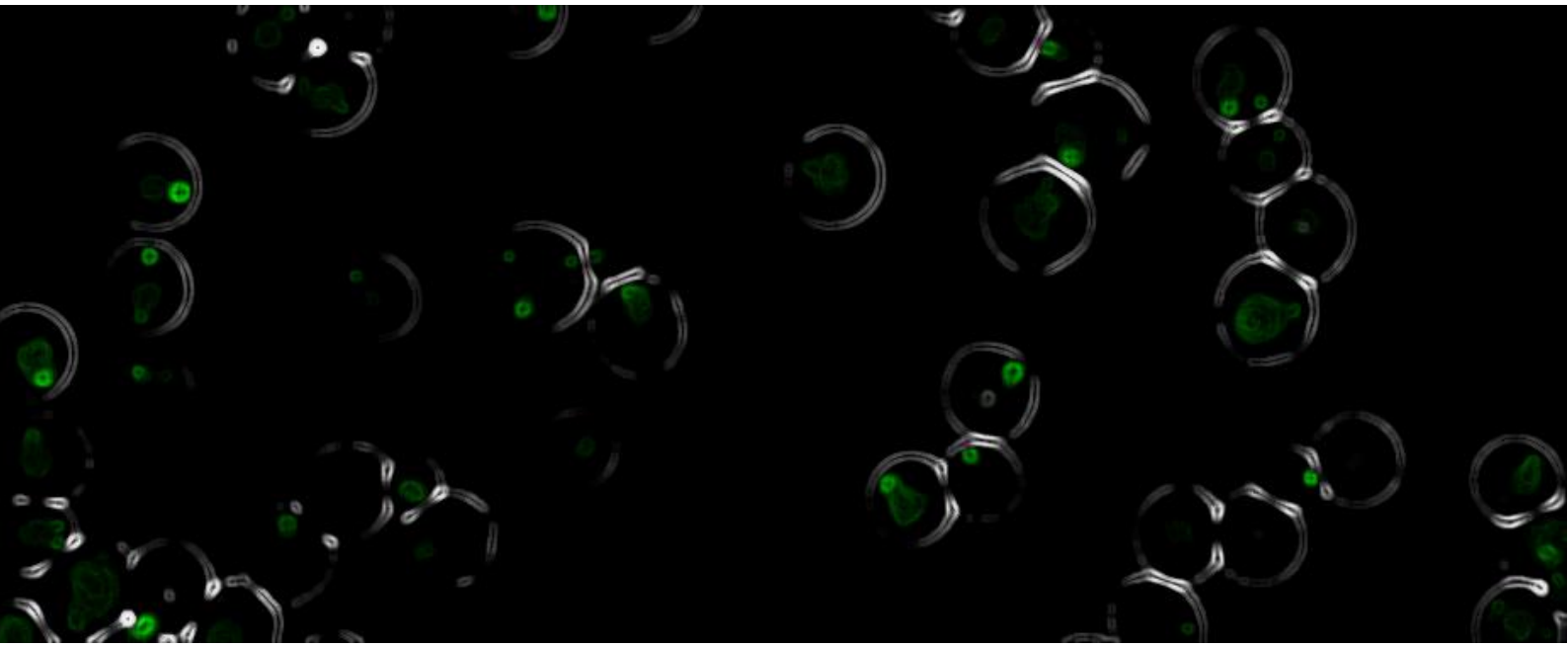

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Cover image. **Edited FM picture.** Pex3-mKate stained peroxisomes (green) in the yeast *Hansenula Polymorpha* grown on glucose. (FM = fluorescence microscopy). Edited with artistic effects in Word. Reference: own image.

SUMMARY

Peroxisomes are semi-autonomous organelles present in all eukaryotes. They are involved in important metabolic pathways. Proteins that are involved in the biogenesis of peroxisomes are called peroxins or PEX proteins. Peroxisomes do not contain their own DNA, so peroxisomal proteins are synthesized elsewhere before being transported to peroxisomes.

This thesis will focus on the transport of proteins destined for the peroxisomal matrix, also called peroxisomal matrix protein import. Several diseases can arise when something goes wrong during peroxisomal matrix import so it is important to understand the mechanism. The exact mechanism of peroxisomal matrix protein import has been disputed for several years. Two main models have existed for about 20 years to explain the mechanism of peroxisomal matrix protein import, until a third model came along in 2022.

Mainly the role of the peroxin PEX5 is disputed in these models, so the three models are described with a focus on the role of PEX5.

It was found that in two models PEX5 enter the matrix of peroxisomes entirely, namely the PEX5 as extended shuttle and PEX5 as cargo accompanier models. In one model PEX5 inserts in the peroxisomal membrane and forms a transient pore, this model is called membrane shuttle model.

The models were compared, and it was concluded that PEX5 most likely enters the peroxisomal matrix completely before being pulled out as described by Skowyra and Rapoport (2022). Although questions on cargo release remain.

It is very exciting to see that new models and ideas on peroxisomal matrix import are arising. This is probably not the end of the story, so this conclusion may change in the future as more research is done.

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LIST OF ABBREVIATIONS

AAA ATPase associated with diverse cellular activities
AO alcohol oxidase
ER endoplasmic reticulum
FCS fluorescence correlation spectroscopy
FM fluorescence microscopy
NPC nuclear pore complex
PDB peroxisome biogenesis disorder
PEX peroxin gene
PEX peroxin protein in mammals
Pex peroxin protein in yeast
PM peroxisomal membrane
PMP peroxisomal membrane protein
PTS peroxisomal targeting signal
PTS1 C-terminal PTS signal
PTS2 N-terminal PTS signal
RING really interesting new gene
TPR tetratricopeptide repeat (binds PTS1)
Ub ubiquitin

INTRODUCTION

Peroxisomes

Peroxisomes are organelles with highly diverse functions (Lazarow, 2023). They are essential for eukaryotic cells to function normally, however, giving a brief overview is difficult as they adapt their function depending on the enzymatic environment (Lazarow, 2023). There are a few functions that are very common. Almost all peroxisomes detoxify hydrogen peroxide with the enzyme catalase and are involved in the metabolic pathway that β-oxidizes fatty acids (Farré et al., 2019).

Peroxisomes are extremely important for human health. It is already known for a long time that mutations in peroxisomal genes can cause diseases that are generally lethal, like Zellweger syndrome (Aksit & van der Klei, 2018) (Kalel & Erdmann, 2018). Recently it has become clear that small deficiencies in peroxisomal functioning also have huge effects on human health, it can lead to cancer and neurodegeneration for example. (Zalckvar & Schuldiner, 2022). Some of these non-lethal diseases occur because the import of proteins into peroxisomes is defect by a gene alteration (Ravindran et al., 2023). Understanding the role of peroxisomal genes is thus very important to get a better grasp on when diseases arise and how they can be prevented and treated.

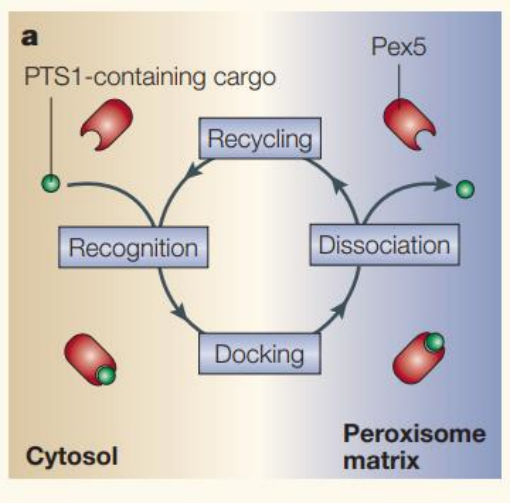

Figure 1. **The peroxisomal matrix protein import cycle.** Pex5 recognizes the PTS1, and they bind. The receptor-cargo complex docks at the peroxisomal membrane. The cargo is released, and the cycle starts again. Reference: Erdmann & Schliebs, 2005.

Peroxisomal matrix protein import

Peroxisomes are semi-autonomous organelles, meaning that they can grow and divide on their own, however their proteins are synthesized elsewhere as they do not contain any DNA (Lazarow, 2023). So, proteins are synthesized in the cytosol and transported to the matrix, the inside of the peroxisomes. Proteins that are targeted to the peroxisomal matrix or membrane have a peroxisomal targeting signal (PTS) (Lazarow, 2023). Although more are hypothesized (Kunze, 2020), generally, there are two signals for peroxisomal matrix import, a C-terminal PTS1 and a N-terminal PTS2 (Lazarow, 2023). For a long time, it was thought that there is an identifiable consensus sequence for these signals (Kunze, 2020). Older papers report (S/A/C)-(K/R/H)-L as the consensus sequence for PTS1 (Erdmann & Schliebs, 2005). Recently it was found that a broad variety of tripeptides can function as a PTS1 signal (Kunze 2020). There are a variety of nonapeptide sequences that can function as the PTS2 signal (Kunze, 2020).

Peroxins are proteins that are involved in the biogenesis of peroxisomes, they are either important for the import of proteins into the matrix of peroxisomes or they are important for the peroxisomal membrane (Lazarow, 2023). The genes for peroxins are known as *PEX* genes, and their proteins are known as PEX proteins in humans and Pex proteins in yeast (Lazarow, 2023). At the moment 37 PEX proteins are known (Jansen et al., 2021). For peroxisomal matrix protein import, a few peroxins play a major role. The PTS signals are recognized by their sequences via peroxin receptors. Typically, the receptor PEX5 recognizes PTS1 and the receptor PEX7 recognizes PTS2 (Farré et al., 2019). A simplified overview of all the proteins imported for peroxisomal matrix protein import can be found in **Table 1**.

Peroxisomal matrix protein import consists of four steps (Kim & Hettema, 2015) (**Figure 1**). The first step is that a receptor, either PEX5 or PEX7 recognizes the PTS in the cytosol (Farré et al., 2019). These receptors sometimes work together with other co-receptors, and other times they work alone (Farré et al., 2019). Secondly, the complex of the receptor, cargo, and possible co-receptors can attach to the peroxisomal membrane at the site of a docking

Table 1. **Simplified overview of all Pex proteins associated with peroxisomal matrix import.** The first column gives the complex that the protein is associated with. The second column gives the peroxin name in yeast and the third column gives the peroxin name in humans if one exists. The last column gives a brief overview of the function of the peroxin, although this might differ a bit per model. Table is reproduced from a combination off the sources Liu et al., (2012) and Kalel & Erdmann, (2018).

PROTEINS	*yeast*	*human*	**FUNCTIONS AND PROPERTIES**
Import receptors	Pex5	PEX5	PTS1 receptor, contains C-terminal TPR domains
	Pex9	PEX5	PTS2 co-receptor, contains Pex7 binding box
	Pex7	PEX7	PTS2 receptor, contains WD domains
	Pex20/Pex18 /Pex21/Pex5	PEX5	PTS2 co-receptor, contains Pex7 binding box
Docking	Pex13	PEX13	Contains SH3 motif
	Pex14	PEX14	Component of the peroxisomal translocon
	Pex17		Associates with Pex14,
	Pex33		Contains an N-terminal domain homology to Pex14
	Pex8		Importomer assembly (cargo release)
	Pex3		Importomer assembly
RING-finger complex	Pex2	PEX2	E3 ligase, receptor ubiquitination
	Pex10	PEX10	E3 ligase, receptor ubiquitination
	Pex12	PEX12	E3 ligase, receptor ubiquitination
Ubiquitin-conjugating complex	Pex4		Ubiquitin-conjugating enzyme, E2
	Pex22		Peroxisomal anchor for Pex4
AAA+ complex	Pex1	PEX1	AAA ATPase for receptor recycling
	Pex6	PEX6	AAA ATPase for receptor recycling
	Pex15	PEX26	Peroxisomal anchor for Pex6

complex (Farré et al., 2019). Where and why is still unclear, but the cargo is released from the receptor, concluding the cycle and the PTS-recognizing receptor is recycled again (Erdmann & Schliebs, 2005).

Different models exist with slightly distinct functions for the PEX proteins involved in peroxisomal matrix protein import. However, most models do not differ all too much, consisting of the same complexes that can be seen in **Figure 2**. In most models the docking site consists of Pex13, Pex14 and Pex17 (Farré et al., 2019), this docking complex recruits the Pex5-cargo complex (Skowyra & Raporort, 2022). Pex2, Pex10 and Pex12 form the RING complex, also called the ubiquitin ligase complex (Farré et al., 2019) (Kim & Hettema, 2015). The docking site together with the RING complex is where the receptor imports or inserts together with the cargo (Farré et al., 2019). Unique to peroxisomal matrix transport is that folded proteins can be imported unlike in the import of mitochondria and the endoplasmic reticulum

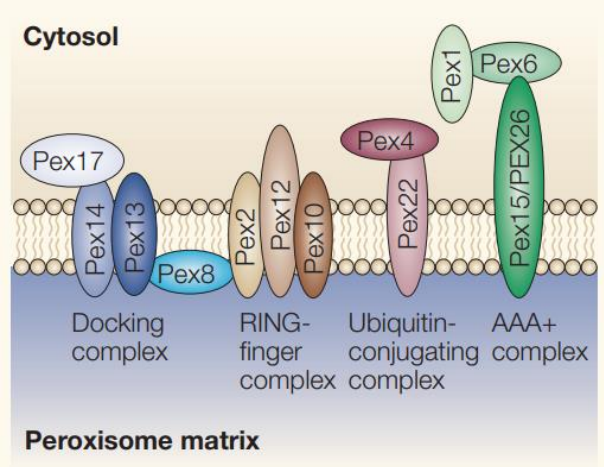

Figure 2. **Complexes involved in peroxisomal matrix import.** The docking complex consists of Pex13, Pex14 and Pex17. The RING-finger complex consists of Pex2, Pex12 and Pex10. Pex8 probably links these two complexes at the matrix site. Pex4 can ubiquitinate and is docked to the membrane via Pex22. The AAA+ complex is proposed to be involved in the final stage of peroxisomal matrix protein import and consists of Pex1, Pex6 and Pex15 or PEX26 in mammalians. Reference: Erdmann & Schliebs, 2005

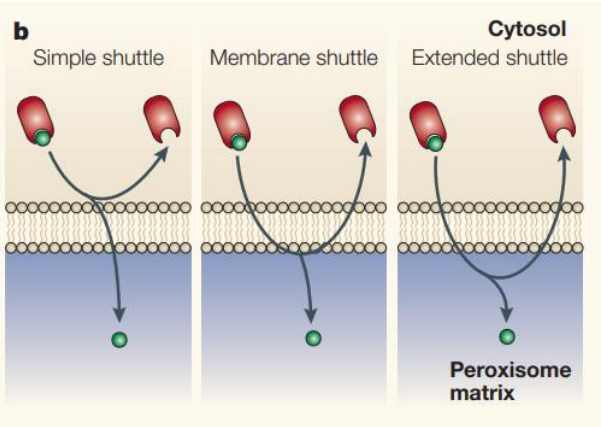

Figure 3. **The peroxisomal matrix protein import models.** In the simple shuttle model, the cargo is released at the cytosolic side. In the membrane shuttle model, the receptor forms a pore complex in the membrane. And in the extended shuttle model the receptor moves through the membrane and release the cargo at the matrix site of the peroxisome. Reference: Erdmann & Schliebs, 2005

(Skowyra & Rapoport, 2022). Pex5 gets ubiquitinated and then exported / disassembled with the exporter consisting of Pex15, Pex1 and Pex6 (Farré et al., 2019) (Kim & Hettema, 2015).

Peroxisomal matrix protein import models

Although, as described above, a broad overview of peroxisomal matrix protein can be given there still is a lot of debate about the details. Especially on where and how imported proteins are released (Erdmann & Schliebs, 2005).

Three main models exist for the mechanism of peroxisomal matrix import (**Figure 3**), the simple shuttle model, the membrane shuttle model, and the extended shuttle model (Erdmann & Schliebs, 2005). The simple shuttle model says that the imported protein releases at the cytosol side of the peroxisome. This model will not be discussed a lot further. The extended shuttle model says that the receptor travels all the way into the peroxisomal matrix before releasing the cargo and then going back out (Erdmann & Schliebs, 2005). This model will be discussed. Along with the membrane shuttle model, that says that the receptor forms a transient pore in the membrane through which the imported protein can travel (Erdmann & Schliebs, 2005). Both models are around 20 years old, and evidence for both still appears.

Recently, a new model appeared by Skowyra & Rapoport (2022) which says that the receptor moves into the matrix with the cargo, and the cargo is only released during export.

PEX5

The role of PEX5 is the most diverse in all the models. The exact mode of operation of PEX5 is a longstanding question (Skowyra & Rapoport, 2022). The docking of PEX5 is a process that is poorly understood, as well as how PEX5 returns to the cytosol (Skowyra & Rapoport, 2022).

A few things about PEX5 are known. It is a soluble receptor (Skowyra & Rapoport, 2022). The function of the N-terminus is poorly defined, but following the N-terminus is the globular tetratricopeptide repeat (TPR) domain (Skowyra & Rapoport, 2022). This domain binds to PTS1. PEX5 does not have a few hydrophobic amino acids following each other, so there is no transmembrane domain (Kunau, 2001).

As described, multiple models on peroxisomal matrix protein import exist to this date. Especially the role of PEX5 is debated. Here three models that exist will be compared and assessed based on their strengths and weaknesses. In the end, the most likely role of PEX5 in peroxisomal matrix protein import will be given.

PEX5 AS EXTENDED SHUTTLE

PEX5 shuttles cargo in peroxisomal lumen

The first model that will be discussed is the extended shuttle model. The model was already proposed in 1995 (Rachubinski & Subramani, 1995). At that time, another very prevalent model existed, the simple shuttle model. (Dammai & Subramani, 2001) The simple shuttle model was first proposed for Pex7 in *Saccharomyces cerevisiae* (Kunau, 2001). Because the location of PEX5 and PEX7 (PTS receptors) is mostly cytosolic this model was proposed (Dammai & Subramani, 2001). In the simple shuttle model, the receptor shuttles between the cytosol and the membrane of peroxisomes (Kunau, 2001). The receptor binds with cargo to the membrane and leaves again without cargo (Kunau, 2001).

This model was extended when evidence arose that the receptor might enter the peroxisomal matrix, giving rise to the extended shuttle model (Kunau, 2001). This model was supported in 2001. Dammai & Subramani showed that PEX5 enters the peroxisomal matrix.

This model, like every other model, proposes that peroxisomal matrix protein import starts with binding of PEX5 to PTS1 forming the receptor-cargo complex in the cytosol. The model also proposes that that the docking site is necessary to give directionally to the receptor-cargo complex (Kunau, 2001). As candidates for the docking site, they suggest PEX13 and PEX14, which is in line with the other models. The idea in the model is that the export of PEX5 is done by the RING finger complex (Pex2, Pex10 and Pex12).

So, in this model, PEX5 functions as an extended shuttle, entering the peroxisomal matrix to deliver cargo.

Experimental confirmation

Dammai & Subramani (2001) did a series of experiments and concluded that PEX5 has operates via the mechanism proposed in the extended shuttle model. A few of these results will be reviewed here. For their experiments they used the human PEX5 protein in different variants of human cells. They used cells with healthy peroxisomes (HeLa) and with a PEX5 deficiency (Ala-T) (Dammai & Subramani, 2001).

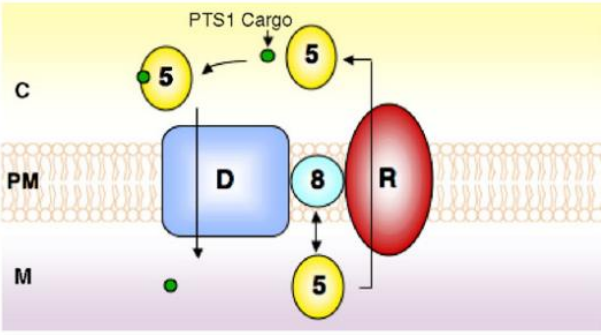

Figure 4. **Model of PEX5 as extended shuttle.** Pex5 binds PTS1 cargo and moves through the docking complex. The cargo is released, although it is not mentioned how. Through interaction with Pex8, Pex5 translocates to the RING complex that exports Pex5 back to the cytosol for another round of import. D: Docking subcomplex, R: RING subcomplex, 5: Pex5, 8: Pex8, C: Cytosol, PM: Peroxisome membrane, M: Peroxisome matrix. Reference: Rayapuram & Subramani, 2006

They showed that in Ala-T cells with a PTS1 signal ended up in the cytosol but with PTS2 they ended up in the peroxisomes, confirming that PEX5 is needed for PTS1 import (Dammai & Subramani, 2001). It was also shown that Pex5 enters the peroxisomal matrix independently of a working PTS2 (**Figure 5**). Pex5 is processed in the matrix, and the processed form increased with time (Dammai & Subramani, 2001).

However, I wonder if they have confidently showed that PEX5 enters the matrix and is not just in the peroxisomal membrane. Proteins can also be modified while partly in the membrane. It is also not possible to see the difference between being on the membrane and in the peroxisome.

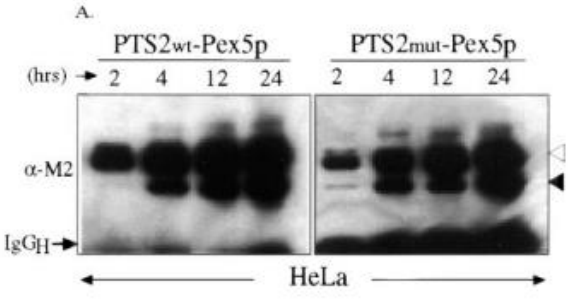

Figure 5. **Immunoblot of Pex5.** The white arrow indicates unprocessed proteins, and the black arrow indicates processed protein. Alpha-M2 is a monoclonal mouse antibody that recognizes Pex5. Reference: Dammai & Subramani, 2001.

They confirmed that PEX5 is used multiple times as processed PEX5 was found on the cytosolic side of the peroxisomal membrane, indicating that it had already gone through at least one round of import (Dammai & Subramani, 2001). The amount of processed PEX5 kept increasing, so it is used in numerous rounds (Dammai & Subramani, 2001).

Cargo release

There is no mention of how the cargo is released in the paper by Kunau (2001) or Dammai & Subramani (2001). Which is a weak point of this model, this is also reported by Skowyra & Rapoport (2022).

However, another paper from 2003 by Wang et al. argued in favour of the extended shuttle model and showed a mechanism of how the cargo could be released from the receptor Pex5

with the help of Pex8. Pex8 contains a PTS1, which explain the binding to Pex5, but there is still binding when PTS1 is removed (Wang et al., 2003). The experiments were done in vitro with proteins from the yeast *Hansenula polymorpha* (Wang et al., 2003). The paper used a technique called fluorescence correlation spectroscopy (FCS). This is a difficult but very sensitive method and is used in the paper for the first time for research on peroxisomes (Wang et al., 2003). Unfortunately, these results have not yet been reproduced again. In the paper found that that Pex8 is a monomer under all pHs and Pex5 can be in different conformations depending on the pH with FCS. When adding Pex8 to PTS1-bound Pex5 they bound fraction went down from 14% to 9% (Wang et al., 2003). Which led them to a model where Pex8 competitively binds which Pex5, causing the cargo to be released (Wang et al., 2003).

PEX5 location

Another weak point of this model is that it cannot be excluded that Pex5 did not enter the matrix entirely. It could also only have been inserted into the peroxisomal membrane (Kunau, 2001). They do show that the N-terminal part of Pex5 gets into the matrix (Dammai & Subramani, 2001) (Skowyra & Rapoport, 2022). It was also shown that Pex7 is present in the peroxisomal matrix, at least partially in *Saccharomyces cerevisiae* (Nair et al., 2004) (Skowyra & Rapoport, 2022). Pex7 binds PTS2. Pex5 is also known to be dependent on Pex7 in mammalian cells for transport across the membrane (Nair et al., 2004). Nair et al. conclude that their results support the extended shuttle model. As in mammals PEX7 and PEX5 form a complex, so if one is present in the matrix the other one is also present (Nair et al., 2004). However, it is still not clear if the entire Pex5 moves inside the matrix (Skowyra & Rapoport, 2022).

Support by other papers

There is a lot of support for this model in other papers, for example in Ozimek et al., 2005, Leon et al. (2006) and Rayapuram & Subramani (2006). It is noticeable that almost all papers that support this model are written by the same authors or are associated with the same authors as the original two papers. Begging the question of whether people do not find the model that they are looking for.

The paper by Ozimek et al. (2006) stands out. In that paper they research the pathway of the AO (alcohol oxidase) protein. AO has an PTS1 receptor and gets imported into peroxisomes (Ozimek et al. 2006). They also argued for the extended shuttle model in their applied approach. Sadly, it seems that they did not find any proof for the model itself, only for the locations of AO.

PEX5 AS MEMBRANE SHUTTLE

PEX5 forms an integral component of translocation channel

The second model discussed here is the membrane shuttle model, also called the transient pore model (**Figure 5**). The model was proposed in 2005 by Erdmann & Schliebs and still papers are presenting results that lead to this model (Ravindran et al., 2023).

In short, the model proposes that the cargo-receptor complex is part of a pore after docking which gets disassembled once the cycle is finished (Erdmann & Schliebs, 2005). Because pores should be specific enough to allow the receptor-cargo complex to diffuse in without bringing in too many cytosolic solutes, Erdmann & Schliebs (2005) defined four characteristics for the pore. The receptor should temporarily be an integral part of the membrane of the peroxisome (Erdmann & Schliebs, 2005). Association with proteins bound for import in peroxisomes should be possible (Erdmann & Schliebs, 2005). Also, the receptor-cargo complex needs to be able to interact with other peroxins to make disassembly possible (Erdmann & Schliebs, 2005). As well as form a complex large enough for the cargo to go through (Erdmann & Schliebs, 2005).

Erdmann & Schliebs (2005) show that the ideal candidate that fulfills all these requirements is Pex5. The transient pore model can be seen in **Figure 6**. The model Erdmann & Schliebs (2005) propose still starts with Pex5 recognizing PTS1 after which it moves to the membrane and inserts into the membrane (Erdmann & Schliebs, 2005). In this model Pex13, Pex14 and Pex7 are suggested to help with the insertion of Pex5 (Erdmann & Schliebs, 2005). The cargo release is thought to happen with competitive binding by Pex8 (Erdmann & Schliebs, 2005). With a

cascade of reactions (mediated Pex2, Pex12, Pex10, Pex4, Pex22) resulting in ubiquitination of Pex5, the disassembly of Pex5 is proposedly triggered (Erdmann & Schliebs, 2005). Pex1 and Pex6 mediate the translocation in this model.

In this model, Pex5 thus functions as a membrane shuttle. It is part of a transient pore that allows the cargo to move over the membrane.

Cargo release

According to Skowyra & Rapoport (2022) cargo release cannot be easily explained by this model. They state that because of the conflicting conclusions in literature, referring to papers still confirming both models, the question of the role of PEX5/Pex5 remains unsolved. This could be seen as a weakness of this model. The paper (Erdmann & Schliebs, 2005) does state that Pex8 is important for cargo release but how they came to that conclusion remains elusive as they did not use a reference when they mentioned this. To my knowledge, they use one reference that mentions the function of Pex8, but this paper described a different function for Pex8. Namely that Pex8 is needed to form the Importomer (Agne et al., 2003).

However, a paper that is not referenced in Erdmann & Schliebs (2005) did state that they found Pex8 to be important for cargo release (Wang et al., 2003). Notably, this paper argues for the extended shuttle model. Wang et al. (2003) conclude that in *H. polymorpha* Pex5-Pex8 complexes are formed, causing the cargo to be released.

PEX5 topology

Pex5 in yeast is seen to be embedded in the peroxisomal membrane (Acevedo & Schliebs, 2006), supporting the Pex5 as membrane shuttle model. Quite a strong interaction with the membrane was found. Acevedo & Schliebs (2006) propose that a small part of the N-terminus (2 kDa) is exposed to the cytosolic site. A part is inserted in the peroxisomal membrane and the C-terminus, including the TPR domain that binds PTS1 is located in the matrix of the peroxisome (Acevedo & Schliebs, 2006). However, no transmembrane segments are found in Pex5 (Skowyra & Rapoport, 2022), so there is not yet an answer how Pex5 gets embedded in the membrane (Acevedo & Schliebs, 2006).

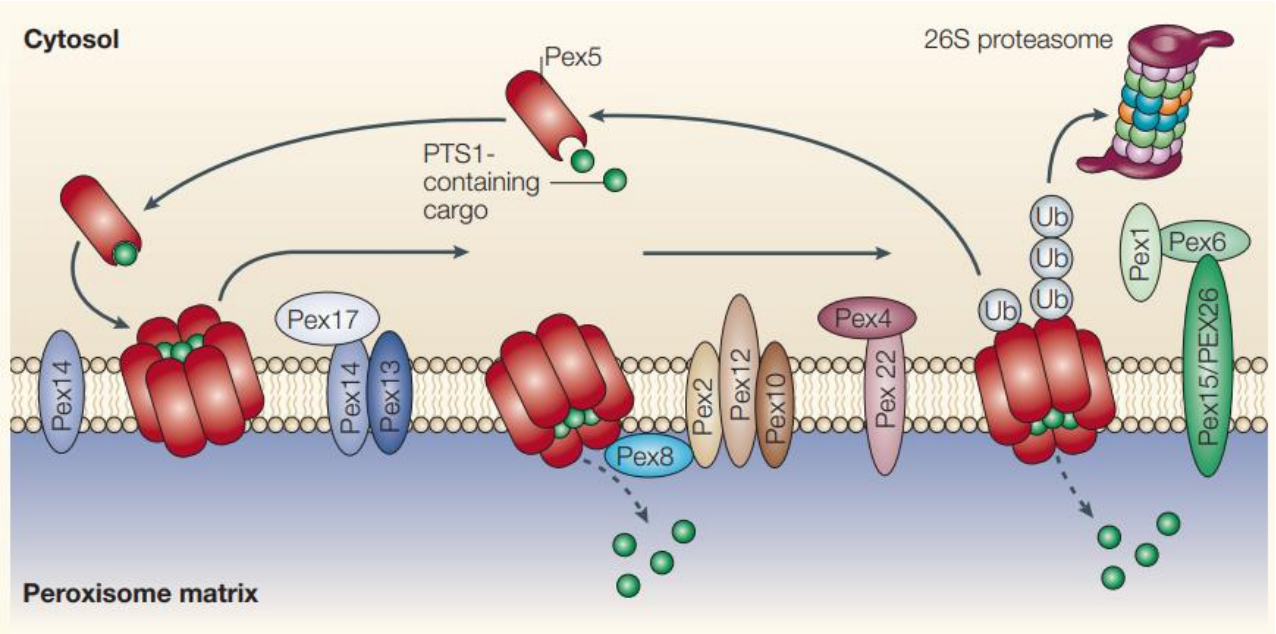

Figure 6. **Model of PEX5 as membrane shuttle.** Pex5 recognizes the cargo by the PTS1, the cargo-receptor complex inserts into the peroxisomal membrane. The docking complex Pex13/Pex13/Pex17 is thought to help with the insertion. Pex8 is thought to bind competitively with Pex5 releasing the cargo in the peroxisomal matrix. Then there is thought to be a cascade of reactions causing the transient pore of Pex5 proteins to be disassembled. This cascade is thought to happen by the RING-finger complex (Pex2, Pex12, Pex10) and the ubiquitin-conjugation complex (Pex22, Pex4). Because of these reactions the Pex5 pore is ubiquitinated and the AAA+ complex (Pex1, Pex6, Pex15) is thought to disassemble the Pex5 pore in an ATP dependent way. Reference: Erdmann & Schliebs., 2005.

Confirmation of functions

All the mechanisms proposed are about the proteins in yeast. Although it is stated that peroxisomal matrix protein import is highly conserved among species (Erdmann & Schliebs, 2005). As far as could be found, the same model has not yet been proposed when doing tests on mammalian cells.

A weakness of the model is that all the functions are proposed, but they still need to be confirmed (Erdmann & Schliebs, 2005). Although a recent paper investigated the functions of several Pex proteins and their findings also led them to the transient pore model (Ravindran et al., 2023).

Ravindran et al. (2023) also state that the transient pore model proposes a mechanism that resembles a mechanism happening in nuclear pore complexes (NPCs). Making the model more biology likely.

PEX5 AS CARGO ACCOMPANIER

PEX5 moves with cargo into peroxisomes

After a lot of debate on existing models, in 2022 a new model arose by Skowyra & Rapoport. They claim that previous models cannot explain how in the lumen the cargo is released from the receptor. With new experiments and results they proposed a mechanism for PEX5. This model looks a lot like the extended shuttle model, however to the model they add a way for the cargo to be released from PEX5 and they also state that the import and export of PEX5 are coupled. The model can be seen in **Figure 7**. Like other models, PEX5 binds to the PTS1 signal on the cargo and the cargo-PEX5 complex goes to the docking site (PEX13 and PEX14) on the membrane of peroxisomes (Skowyra & Rapoport, 2022). The next step is that the PEX5-cargo complex is translocated over the membrane. Skowyra & Rapoport (2022) show that the complex stays in the peroxisomal matrix because PEX14 has high affinity with PEX5 on the matrix side. Through the RING complex PEX5 starts to export out of the matrix, PEX5 is ubiquitinated at the cytosolic side, after ubiquitination PEX5 gets pulled to the cytosol by PEX1/PEX6 AAA ATPase, during the extraction PEX5 is unfolded, causing the cargo to be released (Skowyra & Rapoport, 2022). PEX5 refolds whence it is in the cytosol and gets

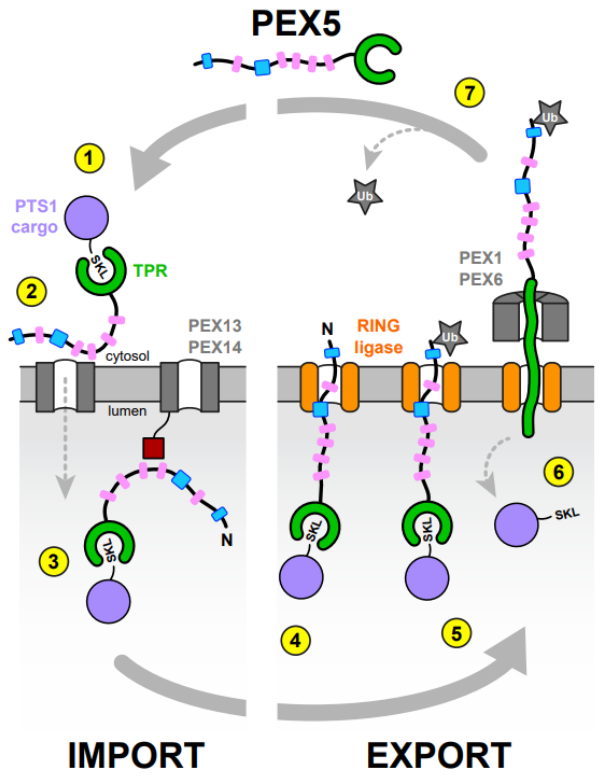

Figure 7. **The peroxisomal matrix protein import models.** 1. With the TPR domain PEX5 can bind PTS1. 2. The cargo-receptor complex goes to the docking complex (PEX13/PEX14). 3. The cargo-receptor complex moves to the peroxisomal matrix. Diffusion back is prevented by PEX14 (red square). 4. PEX5 starts exporting through the RING complex. 5. PEX5 gets ubiquitinated. 6. PEX5 is pulled out with the AAA+ complex (PEX1 and PEX6) causing unfolding and release of the cargo. 7. PEX5 refolds in the cytosol and is deubiquitinated. Reference: Skowyra & Rapoport, 2022.

deubiquitinated (Skowyra & Rapoport, 2022). Then PEX5 can be bound to a PTS1 signal again and the cycle can start again (Skowyra & Rapoport, 2022).

In this model, PEX5 thus moves from the cytosol completely into the matrix with cargo and defolds during export causing the cargo to be released.

PEX5

Skowyra & Rapoport (2022) show that the unstructured region of PEX5 at the N-terminal side is important for import of PTS1 cargo because of the amphipathic helices and

pentapeptide motifs present there. They also show that PEX5 has a transmembrane topology during cycling where the N-terminus faces the cytosol (Skowyra & Rapoport, 2022). They determined that the N terminus does not always face the cytosol, so the N terminus of PEX5 has been in the peroxisomal matrix. That PEX5 has been in the matrix can be seen in **Figure 8**. Skowyra & Rapoport (2022) used two proteins with a PTS2 signal that bind to the N-terminal region of PEX5. On these proteins there is a cleavage site for the TYSND1 enzyme that they kept intact in half of the samples (with the scissor symbol). TYSND1 is naturally present inside the peroxisomal matrix (Skowyra & Rapoport, 2022). It can be seen that in the extract (the cytosol) all the proteins remain in intact. The proteins with an intact cleavage site were cut inside the peroxisomes (lane 5 and 7) so PEX5 has been in the peroxisomal matrix or at least the N-terminal part has been there (Skowyra & Rapoport, 2022).

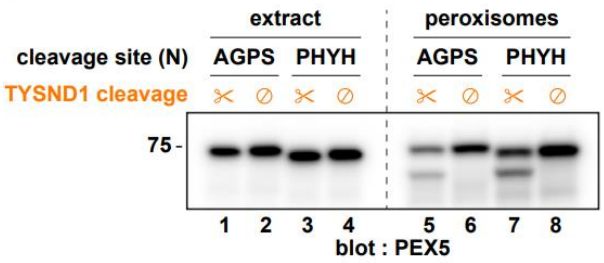

Figure 8. **The N-terminus of PEX5 goes through the peroxisomal matrix.** Alkylglycerone phosphate synthase (AGPS) and phytanyl-CoA hydroxylase (PHYH) are proteins with a PTS2 signal. TYSND1 is an endogenous enzyme that cleaves these proteins in the peroxisomal matrix. The proteins were mutated such that the TYSND1 cleavage site is still intact but the PTS2 targeting function is disabled. However, in the lanes without the scissors the cleavage site was not intact. The peroxisomes were isolated and immunoblotted for PEX5. Reference: Skowyra & Rapoport, 2022.

Next it was tested whether just the N-terminus of PEX5 as a whole enters the peroxisomal matrix and it was found that this was the case (See Skowyra & Rapoport, 2022).

Cargo release

Because they showed that the entire PEX5 proteins enter the peroxisomal matrix, they also need a pathway for PEX5 to be exported to complete their model. The paper does not seem to give their own results as to why PEX5 is exported by PEX1 and PEX6. They hypothesize that it is likely that the TPR domain unfolds during export, causing the cargo to be released. This model is the first to give a clear explanation on how the cargo is released in the matrix, but the evidence seems to still be a bit weak. The fused a nanobody to the TRP domain, the nanobody is unfolded so they conclude it is likely the TPR domain is unfolded as well (Skowyra & Rapoport, 2022). Although this is a likely mechanism, more research should be done on it.

Nuclear pore resemblance

At the end of the paper, there are still a few questions about peroxisomal matrix protein import. The docking of the PEX5-cargo complex is still not completely clear (Skowyra & Rapoport, 2022). Another question that remains is how PEX5 translocates across the membrane without transmembrane regions (Skowyra & Rapoport, 2022).

The authors tried to answer that second question in a follow up paper, where they state that peroxisomal matrix protein import happens through a nuclear pore-like phase (Gao et al, 2022). In this paper it is proposed that PEX13 forms a mesh in the peroxisomal protein through which PEX5 can travel (see Gao et al., 2022). They found that PEX13 contains a YG domain that can cause gelation and confirmed that PEX5 can travel through this gel.

DISCUSSION / CONCLUSION

Here three models that exist on the mechanisms of peroxisomal matrix protein import are described, with a focus on the role of PEX5/Pex5.

There are several weaknesses and strengths to all the models. Both the models were PEX5 functions as an extended shuttle or as a membrane shuttle cannot explain how the cargo is released in the peroxisomal matrix. Although the model was PEX5 functions as a cargo accompanier claims that they can explain how cargo is released, there is not yet a lot of evidence for this. The extended shuttle model does get a lot of support from other papers. The membrane shuttle model gets support from a very recent paper as well and the cargo

accompanier model seems to have the least open questions left. However, no model seems to be able to answer every question.

Before delving into the question of what the most likely model is, it is notable that to my knowledge, almost all papers that concluded a certain model were written by the same author(s). This begs some questions. Could it be possible that the authors found what they were after or do certain techniques used by the different labs lead to the different conclusion? Although a limitation of this study was that probably not all relevant papers have been read, so important insights might be missing.

A possibility that I have not yet seen anywhere is that the different mechanisms exist in different species or under different conditions. The membrane shuttle model has only been seen in yeast, while the cargo accompanier model has only been seen in humans.

However, after considering all these models with the knowledge that was gathered, it seems likely that PEX5 enters the matrix entirely, probably in the fashion described by Skowyra & Rapoport (2022). Although a few questions remain on the cargo release. The extended membrane models make a compelling case that Pex8 causes the cargo to be released. Skowyra & Rapoport never mention Pex8, because a human equivalent does not exist and the experiments on Pex8 were done in yeast. It could thus be possible that the mechanism for cargo release is different in yeast and mammals.

The question of docking and how PEX5 transports over the membrane is also still not completely resolved. Gao et al. (2022) claim that import happens through a nuclear pore like phase. Notably, this is the same claim that the membrane shuttle model made even though that model differs the most from this model. There is a difference in the claim. Ravindran et al. (2023) from the membrane shuttle model claim that Pex5 forms a nuclear pore like structure with Pex13 and Pex14. Gao et al. (2022) claim that the nuclear like pore is made by PEX13 through which PEX5 can travel. The results from Gao et al. (2022) do seem to explain why PEX5 can enter peroxisomes while having zero transmembrane regions.

In conclusion, PEX5 probably imports as described by Gao et al. (2022) and Skowyra & Rapoport (2022). How the cargo is released is still not completely clear, and then PEX5 is probably exported as described by Skowyra & Rapoport (2022), at least in humans.

The role of PEX5 in peroxisomal matrix import remains a relevant questions, and with the new interest and with new models, I look forward to new research that will be done and new insights that will be discovered.

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