The peroxisomal protein importomer: a bunch of transients with expanding waistlines

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Peroxisomes can import large multimeric protein complexes and even 9 nm gold particles decorated with peroxisome targeting signals. They achieve these feats of protein passage using a distinctive translocon whose highly dynamic aqueous pore can expand to accommodate the increasing girths of different peroxisome receptor-cargo complexes.

Peroxisomes have always been the bad boys of the organelles, not following conventions and doing things their own way. They can grow and divide just like mitochondria and chloroplasts, but they can also be made de novo from the endoplasmic reticulum[1]. And like mitochondria and chloroplasts, peroxisomes can import proteins from the cytosol. But here too peroxisomes show their rebellious nature. Peroxisomes like to translocate fully folded and assembled proteins across their membranes and even allow for the import of proteins lacking a peroxisomal-targeting signal that piggyback onto proteins with a peroxisomal-targeting signal[2]. Peroxisomes can even specifically import nondeformable 9 nm gold particles decorated with a peroxisome-targeting signal[3]. And because peroxisomes can move proteins across their membrane unconventionally, the long-standing question has been how they achieve this feat. Now on page XXX of this issue, Meinecke and coworkers have provided an answer with the finding that the peroxisomal matrix protein Pex5p and its partner Pex14p forms a ion-conducting channel that can expand in diameter when cytosolic receptor-cargo complex is encountered[4].

Over the years, several models for moving unconventional protein cargoes across the peroxisomal membrane have been proposed, including vesicular fusion and a type of pinocytosis; in the latter case, proteins bound to the peroxisomal membrane would access the peroxisome interior by invagination of the peroxisomal membrane, followed by budding of a vesicle containing peroxisomal protein cargo and eventual release of the cargo into the peroxisomal matrix by dissolution of the vesicle membrane[5]. However, most models have proposed the existence of an aqueous pore that crosses the peroxisomal membrane and is transient in nature[6,7]. In fact, large conductance channels have been identified in the membranes of mammalian peroxisomes[8], but because the proteins that make up the pores of these channels are unknown and because no link has been demonstrated between these channels and peroxisomal matrix protein import, their role, if any, in moving proteins across the peroxisomal membrane has not been confirmed.

Most peroxisomal matrix proteins end in a carboxyl-terminal tripeptide of Ser-Lys-Leu called the peroxisome-targeting signal type 1 (PTS1). PTS1 proteins are recognized by the PTS1 receptor, Pex5p. Pex5p is a cycling receptor that recognizes PTS1 cargo in the cytosol, docks at the peroxisomal membrane, integrates into the membrane to deliver its cargo, then recycles to the cytosol for another round of PTS1 protein import. Docking of the Pex5p-PTS1 cargo complex at the peroxisomal membrane occurs via its interaction with Pex14p or Pex13p, which form the core components of the docking machinery that also includes a protein of undefined function, Pex17p. The soluble matrix protein Pex8p is the final component of the translocation pathway and acts to link the docking complex to the Pex5p export machinery[9].

Although we have known for a long time that peroxisomes import soluble proteins post-translationally from the cytosol and the proteins involved in this process have been identified, we have had a fuzzy image of how peroxisomes actually achieve matrix protein import. Now, Meinecke and colleagues have focused this image for us. They have reconstituted the peroxisomal importomer and shown that it is a gated ion-conducting channel forming a large and highly dynamic pore. Crucial to their eventual success in reconstituting a functional peroxisomal importomer was the previous proposal that PTS receptors, including Pex5p, form a key component of a transient pore at the peroxisomal membrane[7]. With this concept in mind, Meinecke and colleagues isolated complexes from yeast peroxisomal membranes by affinity purification of a tagged version of Pex5p. These complexes

were subjected to size exclusion chromatography, and immunoblot analysis of fractions detected the presence of Pex5p over a broad range of molecular masses from the size of monomeric Pex5p to extremely large complexes of 800 kDa and more. These complexes contained to a greater or lesser extent: the Pex proteins shown to be involved in Pex5p docking, including Pex13p, Pex14p and Pex17p; the peroxisomal export machinery components Pex10p and Pex12p; Pex8p which links the docking complex with the export machinery; and Mdh3p, a peroxisomal form of malate dehydrogenase that contains a PTS1 and is a cargo of Pex5p.
Three complexes were defined. Complex III has a molecular mass greater than 800 kDa and contains all of these proteins and was assumed to represent the assembled peroxisomal importomer. Complex II has a molecular mass spanning a range of 600 to 800 kDa and consists of Pex5p associated with Pex14p and its partner Pex17p but lacks cargo protein and the Pex proteins of the export machinery, Pex10p and Pex12p. Complex I does not contain the docking proteins Pex13p and Pex14p, the export proteins Pex10 and Pex12p, or Mdh3p cargo and was considered to represent an early step in the import pathway.

When all three purified complexes were reconstituted into liposomes and analysed for pore-forming activity using the planar lipid bilayer technique[10], nothing much happened. Ion channel activity was detected with complexes II and III, but channels formed only sporadically and displayed a variety of conductance states and ion selectivities. The authors reasoned that the observed heterogeneity in channel activity was the result of the Pex5p-dependent pore being a transient structure that continuously assembles and disassembles. They had to design a more stable pore. How to do this?

The authors eliminated Pex8p, thereby preventing the association of the docking complex with the Pex5p export machinery and stabilizing the translocon. They also prevented the interaction of an alternative peroxisomal matrix protein import pathway, the PTS2 pathway, with the PTS1 pathway by deleting the *PEX18* and *PEX21* genes which encode a pair of chaperones that facilitate PTS2-dependent protein targeting to the peroxisome. The PTS1 and PTS2 import pathways converge at the peroxisomal membrane[7]. Removing PTS2 import elements provided a more homogenous and better defined preparation of purified Pex5p-dependent import pore.

As expected, Complex III shifted to a much lower molecular mass because of the loss of export peroxins, and Complex II, because of its lack of export peroxins, retained a molecular mass of approximately 700 kDa as Complex II*. Complex II* contained Pex5p, Pex13p and Pex14p, reduced levels of Pex17p as compared with wild-type Complex II, and no Mdh3p cargo. Reconstitution of Complex II* into liposomes and their incorporation into planar lipid bilayers resulted in the formation of channels that demonstrated distinct gating with a mean of the main conductance state of 85 pS. This corresponds to a rather unimpressive pore diameter of 0.6 nm --- much less than the diameter of a protein translocating channel pore of mitochondria or chloroplasts[11,12], which translocate unfolded proteins but can neither accommodate nor import the large, folded and multimeric proteins that a peroxisomal translocon moves across the peroxisomal membrane. Attempts at opening the peroxisomal channel by providing either synthetic or natural PTS1-containing cargoes were unsuccessful. However, when reconstituted Complex II* was presented with purified Pex5p-cargo complex and its ion channel activity measured, the situation changed dramatically. Channel gatings with a mean value of 760 pS and a pore diameter of 2.8 nm were now observed, as were maximal observations of 1150 pS and a pore diameter of approximately 3.8 nm. Now these were much more respectable pore diameters for a peroxisomal translocon, but they were still insufficient for the translocation of other than small folded proteins. How then might the channel pore allow passage of large oligomerized peroxisomal proteins?

Meinecke and colleagues postulated that the Pex5p-inducible channel can transiently expand to accommodate large peroxisomal protein cargo and indeed this was the case. They isolated a 750-kDa Pex5p cargo complex containing the peroxisomal matrix enzyme fatty acyl-CoA oxidase (Fox1p) and preincubated it with proteoliposomes before analyzing for pore forming activity. Impressively, conductance states corresponding to pore sizes of more than 9 nm were occasionally observed, demonstrating that the peroxisomal translocon could widen enough to accommodate the 9 nm PTS1-decorated gold particles previously observed to be imported into peroxisomes[3]. Subsequent experiments analysing the connectivity of gating events showed that the peroxisomal

import pore consists of one highly dynamic channel, in distinct contrast to the protein import pores of other organelles which have been reported to encompass multiple coupled pores within one complex. In effect, the authors' data suggest that the peroxisomal import pore shifts between two different states: an inactive, stand-alone membrane complex and an active, dynamic, and highly conductive translocation pore when this membrane complex associates with Pex5p linked to its cargo (Fig. 1).

This discovery, as is always the case, raises several questions: What are the proteins that form the flexible portion of the channel and how do they flex? What is the channel response to PTS2 cargo and the PTS2 receptor, Pex7p? How many receptor-cargo complexes can the channel accommodate at the same time? Can an in vitro system be established that allows for the true import of a peroxisomal matrix protein into the lumen of an artificial lipid vesicle? Whatever the answers to these questions will be, there is no denying that the distinctive nature of the peroxisomal translocon is in keeping with the nonconformist reputation of that bad-boy organelle, the peroxisome.

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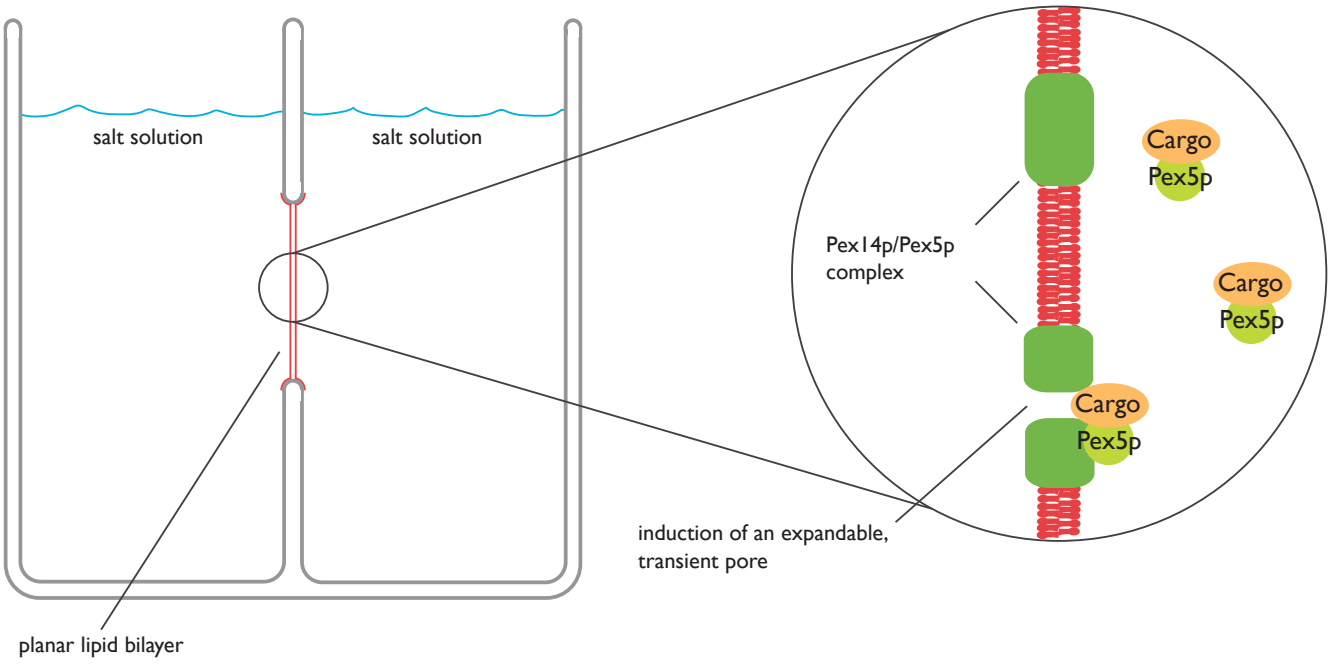
Figure legend

Figure 1. A planar lipid bilayer assay can be used to demonstrate the induction of an expandable and transient peroxisomal translocon channel by cargo-laden Pex5p. The peroxisomal matrix protein importomer can be reconstituted when membrane-anchored Pex14p/Pex5p complex encounters soluble Pex5p bound to cargo. To accomodate the various types of cargo destined for the peroxisomal matrix, the importomer channel can expand to 9 nm or more in diameter. These findings explain how the peroxisome can import fully folded and even oligomeric protein complexes into the peroxisomal matrix.

Figure 1.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