Review

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Good things come to those who bait: the peroxisomal docking complex

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Abstract: Peroxisomal integrity and function are highly dependent on its membrane and soluble (matrix) components. Matrix enzymes are imported post-translationally in a folded or even oligomeric state, via a still mysterious protein translocation mechanism. They are guided to peroxisomes via the Peroxisomal Targeting Signal (PTS) sequences which are recognized by specific cytosolic receptors, Pex5, Pex7 and Pex9. Subsequently, cargo-loaded receptors bind to the docking complex in an initial step, followed by channel formation, cargo-release, receptor-recycling and -quality control. The docking complexes of different species share Pex14 as their core component but differ in composition and oligomeric state of Pex14. Here we review and highlight the latest insights on the structure and function of the peroxisomal docking complex. We summarize differences between yeast and mammals and then we integrate this knowledge into our current understanding of the import machinery.

Keywords: docking complex; peroxins; peroxisomal biogenesis; peroxisomal import; peroxisomal translocon.

Introduction

Peroxisomes are highly versatile eukaryotic organelles that encapsulate particularly reactive metabolic pathways and contribute significantly to cellular redox balance and detoxification due to reduction of reactive oxygen species (Imanaka and Shimozawa 2019; Tolbert 1971; van den Bosch et al. 1992). They carry out a number of central and conserved processes such as β-oxidation of very long-chain fatty acids, metabolism of cholesterol and D-amino acids, oxidation of alcohols, synthesis of plasmalogens and bile acids, and polyamine catabolism (Dinis-Oliveira 2016; Imanaka and Shimozawa 2019) and have been recently discussed as important regulators of immune responses in human (Ferreira et al. 2019; Ganguli et al. 2019). Among the eukaryotes, some organisms have developed specialized peroxisomes. Trypanosomatid parasites harbor their glycolysis pathway in glycosomes (Haanstra et al. 2016), while plants require glyoxisomes for parts of their photorespiration (Tolbert 1971). Peroxisomes are highly adaptable to environmental conditions and more recently were shown to play vital roles in cellular stress response (Okumoto et al. 2020). There is also increasing evidence that impaired peroxisomal function is intertwined with age-related diseases (Cipolla and Lodhi 2017; Fransen et al. 2013) such as cancer (Dahabieh et al. 2018), diabetes and neurodegeneration (Imanaka and Shimozawa 2019). Defects of genes related to peroxisomal biogenesis and/or peroxisome-encapsulated processes lead to severe and life-threatening human diseases, often resulting in an early death (Honsho et al. 2020; Imanaka and Shimozawa 2019; Wanders and Waterham 2005; Waterham and Ebberink 2012).

Peroxisomes do not contain genetic information nor ribosomes (Lanyon-Hogg et al. 2010; McNew and Goodman 1994). However, it has been recently shown that ribosomes translating mRNA transcripts for proteins destined to peroxisomes are in close proximity to the peroxisomal membrane (Dahan et al. 2022). This supports the scenario that peroxisomal proteins might be synthetized and subsequently imported in a highly orchestrated and locally confined manner.

The import process of peroxisomal soluble enzymes in the lumen (matrix) of the organelle is cyclic and involves in general three major steps: cargo recognition, cargo translocation and receptor recycling. In this review, we will shortly summarize the current knowledge on the composition of the peroxisomal import machinery and then emphasize in particular on recent developments regarding

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the events taking place at the peroxisomal membrane during cargo-receptor-docking prior cargo translocation. In the past decades, yeasts have evolved as one of the most well established model organisms to study these processes, mainly because peroxisome proliferation can be easily induced by simply changing the carbon source (Evers 1991; Veenhuis et al. 1976). Here, we will therefore describe the respective pathways in yeast and highlight substantial important differences between the yeast and mammalian import machineries.

The peroxisomal import cycle in yeast and mammals

Cargo recognition

Soluble peroxisomal receptors, in baker's yeast *Saccharomyces cerevisiae* (*Sc*), recognize their cargo proteins in the cytosol via the different Peroxisomal Targeting Signals (PTSs) (Gould et al. 1989; Klein et al. 2002; Kempiński et al. 2020; Swinkels et al. 1991). The majority of peroxisomal matrix enzymes carry a C-terminal PTS1 tripeptide and are recognized by the cytosolic receptor ScPex5 (Gould et al. 1989). Recently, two additional PTS1 cargo proteins, Pxp1 and Pxp2, have been identified. They contain the C-terminal signaling sequences PRL and VKL, respectively, expanding thus the range of putative peroxisomal enzymes and the PTS1 consensus sequence to [S/A/H/C/E/P/Q/V]-[K/R/H/Q]-[L/F] (Nötzel et al. 2016). The PTS1 peptide of the cargo binds thereby to a characteristic groove, formed by two clusters of tetratricopeptide repeats (TPRs) at the C-terminal half of *Sc*Pex5 (Gatto et al. 2000; McCollum et al. 1993). TPRs are well characterized scaffolds implicated in protein-protein interactions and assembly of large protein complexes (Zeytuni and Zarivach 2012) and also present in Tom20 and Tom70, of the mitochondrial import machinery (Baker et al. 2007). Recently, in oleic acid grown yeast cells, *Sc*Pex9, which is a *Sc*Pex5 paralogue, was also shown to join the PTS1 pathway and act as cytosolic receptor (Effelsberg et al. 2016; Yifrach et al. 2016). Cargo specificity was limited however to three peroxisomal proteins, namely *Sc*Mls1, *Sc*Mls2 and *Sc*Gto1 (Effelsberg et al. 2016; Rudowitz et al. 2020; Yifrach et al. 2016).

Several cargo enzymes are alternatively destined to the peroxisomal matrix via an N-terminal PTS2 nonapeptide ([R/K]-[L/V/I]-[X]5-[H/Q]-[L/A]) recognized by the receptor *Sc*Pex7 (Figure 1), but so far, this target signal has been identified in the sequence of only few enzymes (e.g. only two in yeast) (Kunze 2020; Swinkels et al. 1991). The *Sc*Pex7-cargo complex is further stabilized under distinct conditions either by the co-receptor *Sc*Pex21p or *Sc*Pex18p enabling recruitment to the peroxisomal membrane (Effelsberg et al. 2015; Purdue et al. 1998) (Figure 1).

The PTS3 is a putative third import route, but less characterized and the associated "targeting signal" has not yet been precisely assigned. A potential PTS3 sequence has only been so far described in yeast as an internal signal patch of cargos lacking PTS1 or PTS2 (Kempiński et al. 2020). Interestingly, the respective cargos bind to the N-terminal domain of *Sc*Pex5 and not in the PTS1-recognising C-terminal TPR-region (Kempiński et al. 2020; Rymer et al. 2018; Yang et al. 2018) similar to the alcohol oxidase (Aox) from *Hansenula polymorpha* (Ozimek et al. 2006). Finally, cargos that generally lack a PTS sequence can be piggybacked and thus co-imported into peroxisomes upon binding to a typical cargo containing a PTS1 or PTS2 target sequence (Yang et al. 2018). Mdh2 binding to Mdh3 (Gabay-Maskit et al. 2020) and Pnc1 (Effelsberg et al. 2015; Kumar et al. 2016; Saryi et al. 2017) associating with the Gpd1 homodimer are characteristic examples for PTS1 and PTS2 piggybacking, respectively (Yang et al. 2018). Furthermore, Pex20 in *Yarrowia lipolytica*, a co-receptor of Pex7 joining the PTS2 pathway, has been shown to import Aox2 and Aox3 via direct binding (Chang and Rachubinski 2019).

Cargo translocation

The soluble cargo-loaded receptor is recruited to the peroxisomal membrane by the docking complex consisting mainly of *Sc*Pex14, *Sc*Pex17 and *Sc*Pex13 (Albertini et al. 1997; Chan et al. 2016; Erdmann and Blobel 1996; Huhse et al. 1998; Smith et al. 1997) (Figure 1B) and subsequently delivers its cargo into the peroxisomal matrix. In this process, it is still controversial whether the receptor *Sc*Pex5 is the main component of the translocation channel or rather passes the membrane through a channel that is formed by the docking complex. In the first scenario, the receptor is expected to undergo a metamorphosis (Lill et al. 2020; Meinecke et al. 2010, 2016) and similar to a pore forming toxin (Chen et al. 2021; Gatsogiannis et al. 2013), directly insert into the membrane, forming a highly dynamic pore facilitating cargo translocation (Erdmann and Schliebs 2005; Gouveia et al. 2000, 2003; Montilla-Martinez et al. 2015). In the alternative model, Pex5 is considered as a cargo-loaded shuttling receptor that is capable to enter the matrix through a central hydrophilic cavity formed exclusively by the docking complex (Dias et al. 2017).

Intriguingly, in contrast to import processes across the membranes of most other organelles, the peroxisomal translocon is highly adaptive and can tolerate cargos of

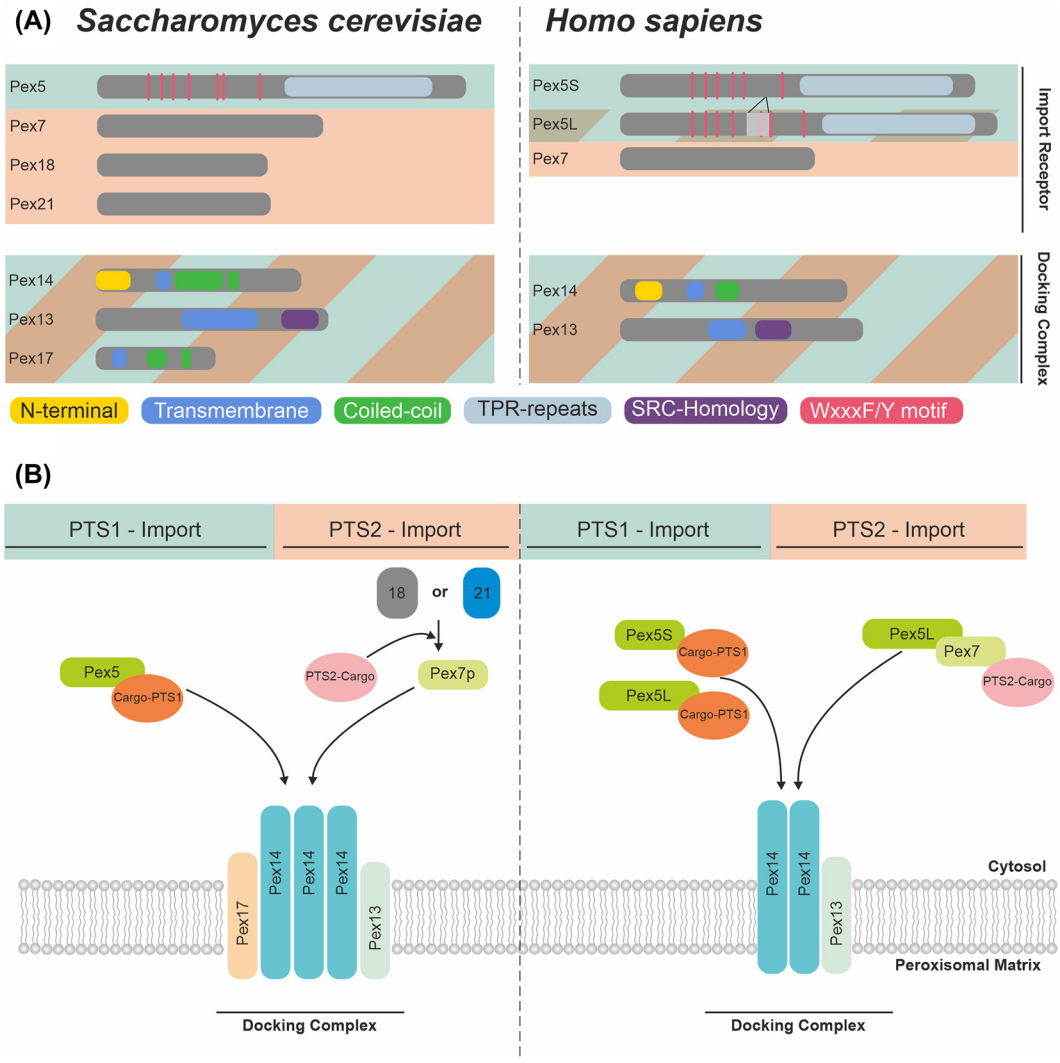

Figure 1: Peroxisomal receptors and membrane docking complexes in yeast and mammals.
(A) Schematic structures of the main components of the yeast (left) and mammalian (right) import receptor- and membrane docking complexes involved in the PTS1 (green) and PTS2 import pathways (red background). Important structural regions are indicated. (B) The import receptors Pex5 and Pex7 are required for peroxisomal targeting of cargos tagged with either PTS1 or PTS2 signals. Mammalian cells harbor in contrast to yeast, at least two Pex5 splice variants HsPex5S (S for short) and HsPex5L (L for long). The longer HsPex5L variant joins as co-factor of HsPex7 in the PTS2 pathway (right), in homology to ScPex18p and ScPex21p in yeast (left). Binding of the cargo-receptor complexes is facilitated via the docking complex consisting of ScPex14, ScPex17 and ScPex13 in yeast (left) or Pex14 and Pex13 in mammals (right).

different sizes (Meinecke et al. 2016; Walton et al. 1992; Yang et al. 2018). The peroxisomal matrix proteins can be thereby imported in their folded or even oligomeric state (McNew and Goodman 1994) similar to translocation events along the nuclear pore (Jovanovic-Talisman and Zilman 2017) or as described for the twin-arginine translocation (TAT) system in bacteria, chloroplasts and archaea (Frain et al. 2019). It should be noted, that the peroxisomal translocon is even capable to import up to 12 nm large artificial substrates (e.g. gold particles, oligosaccharides), as long as they are tagged with a PTS1-peptide (Walton et al. 1995; Yang et al. 2018).

Receptor recycling

Upon membrane insertion of the receptor *Sc*Pex5 and successful cargo-translocation, the receptor is ubiquitinated and further processed by the peroxisomal exportomer. Briefly, ubiquitination of *Sc*Pex5 involves the RING-complex, composed of *Sc*Pex2, *Sc*Pex10 and *Sc*Pex12 (Platta et al. 2004, 2007; Schliebs et al. 2010). Mono-ubiquitination induces recycling of the receptor and requires in addition the E2-enzyme *Sc*Pex4 and its activator *Sc*Pex22, whereas poly-ubiquitination involves the E2-enzyme ScUbc4 and primes the receptor for proteasomal degradation. A recent cryoEM

structure of the RING-complex suggests the formation of a membrane-embedded channel by *Sc*Pex2, *Sc*PEx10 and *Sc*Pex12 for the ubiquitination and retro-translocation of the receptor back to the cytosol (Feng et al. 2022). (poly)-Ub-Pex5 is then further exported from the membrane by the type II ATPAse complex *Sc*Pex1/*Sc*Pex6, that is anchored to the membrane by *Sc*Pex15 (Blok et al. 2015; Ciniawsky et al. 2015; Grimm et al. 2012; Platta et al. 2007; Tan et al. 2016). Notably, ATP is not required for the cargo import itself but rather for receptor recycling, supporting the notion of an export -driven import mechanism (Schliebs et al. 2010; Schwerter et al. 2017).

The mammalian docking process

Most differences in the composition of the respective assemblies in human and yeast are rather restricted to the receptor and docking complexes. In particular, in humans, there are two splice variants of the *Homo sapiens* (*Hs*) receptor *Hs*Pex5, *Hs*Pex5L for long and *Hs*Pex5S for short lacking 111 bp of exon 8 in the PEX5 gene (Braverman et al. 1998) (Figure 1A).

Both isoforms are capable to ferry cargos with the PTS1 signal and target them to the peroxisomal matrix (Braverman et al. 1998). The minority of the mammalian peroxisomal matrix proteins carrying the PTS2 signal, form first a complex with *Hs*Pex7 which is further targeted to the peroxisomes by *Hs*Pex5L (instead of *Sc*Pex18 and *Sc*Pex21 in yeast) (Braverman et al. 1998) (Figure 1B). The mammalian peroxisomal docking complex is also lacking the peripheral component *Sc*Pex17. The events of receptor/cargo docking, cargo translocation and receptor recycling are expected however to follow in general similar mechanistic principles in mammals and yeast. Important differences of the individual components are discussed in the subsequent sections.

Our focus will be on the peroxisomal docking complex, a crucial "checkpoint" for the receptor/cargo-complex at the peroxisomal membrane and in particular, on its key component Pex14 and its multifaceted role in receptor-cargo recognition.

Pex14 - the core component of the docking complex

*Sc*Pex14, a 38.4 kDa protein containing 384 residues (Albertini et al. 1997), is according to PaxDB (Wang et al. 2012) the most abundant peroxisomal membrane component (Barros-Barbosa et al. 2019). Its primary sequence consists of four distinct structural regions (Figure 2A). The N-terminal domain (N) is encoded by the residues 1 to 58

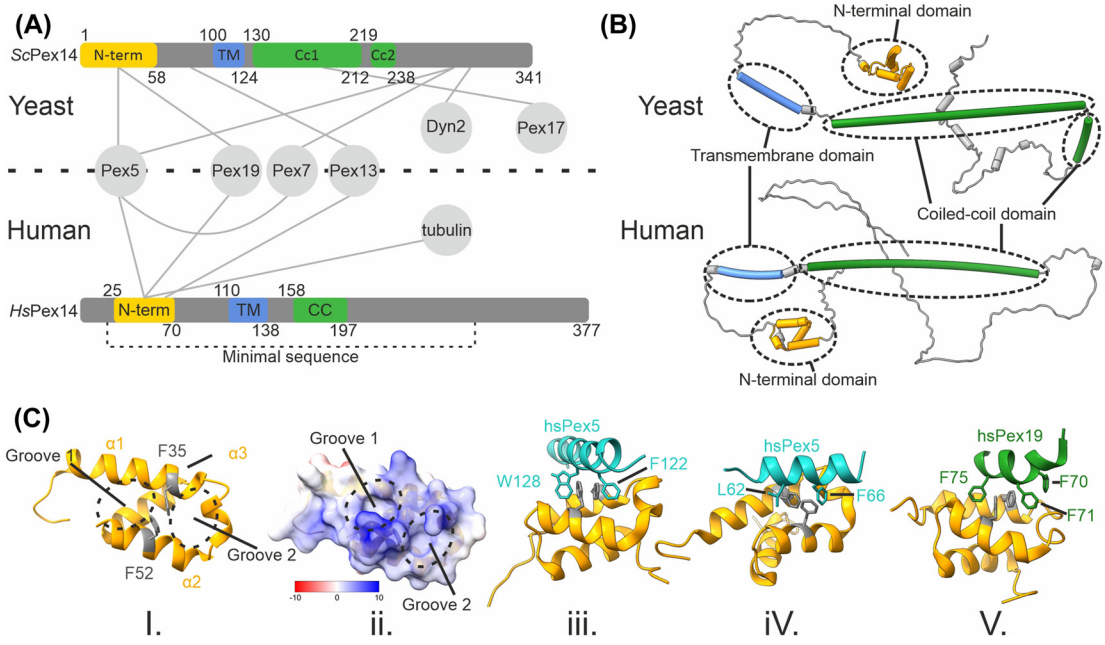

Figure 2: Pex14 – the docking complex core component.
(A) Schematic structure of ScPex14 (upper), HsPex14(lower) and mapped interaction sites with additional proteins. N-terminal domain (N, yellow), transmembrane domain (TM, blue), coiled-coil region (Cc, green) and unstructured regions (grey). (B) Secondary structure predicted via Alphafold2 of the human (AF-O75381-F1) and yeast (AF-P53112-F1) Pex14 isoform. Color legend: N-terminal domain-orange, transmembrane domain-blue, coiled-coil domain-green. (C) Structure of the mammalian Pex14(N) domain as cartoon (i. PDB 3FF5) and electrostatic surface (ii. PDB 3FF5), bound to Pex5-WxxxF-motif (iii. PDB 2W84), bound to Pex5-LxxxF-motif (iv. PDB 4BXU) and bound to Pex19 (v. PDB 2W85). The hydrophobic grooves 1 and 2, separated by F35 and F52 (grey), display an ideal binding site for the shown proteins (iii.-v.).

and connected via a 42 residue long flexible linker to a putative single transmembrane span (TM), an α-helix at residue 100 to 124 (Chan et al. 2016; Lill et al. 2020). Beyond the membrane, a short linker and two coiled-coil (CC) domains are predicted from residues 130 to 212 and 219 to 238, respectively (Chan et al. 2016; Lill et al. 2020). The C-terminal domain is predicted as mainly unstructured and highly flexible.

The *Hs*Pex14 primary structure encodes its N-terminal domain from residue 25 to 70, a TM α-helix from 110-138 (blue) and a single extended coiled-coil domain at residue 158–197 with flexible unstructured peptides (grey) in between (Oliveira et al. 2002) (Figure 2A). Whole protein structure predictions of the yeast (AF-P53112-F1) and human (AF-O75381-F1) Pex14 structure by Alphafold 2 (Jumper et al. 2021; Varadi et al. 2022) confirms the previous overall predicted domain organization (Figure 2B).

The N-terminal domain is considered an important interaction module of Pex14 and the peroxisomal docking complex. It contains binding sites for the PTS receptors (Williams et al. 2005), the chaperone of newly synthetized peroxisomal membrane proteins Pex19 (Neufeld et al. 2009; Sacksteder et al. 2000) and for β-tubulin (exclusively in *Hs*Pex14) (Figure 2A).

The structure of the highly conserved *Rn*Pex14(N) from rat has been solved using X-ray crystallography (Su et al. 2009) (Figure 2Ci. and ii.) and interactions of its human homologue *Hs*Pex14(N) with *Hs*Pex5 and *Hs*Pex19 have been well characterized by NMR spectroscopy (Neufeld et al. 2009; Neuhaus et al. 2014) (Figure 2Ciii. and v.). The available structures unravel a small globular bundle of three alpha-helices, namely α1, α2 and α3 (Figure 2C). Helices α1 and α2 show an antiparallel-arrangement and are connected by a short helical turn. Helix α3 is positioned diagonally, capping the helical pair of α1 and α2 (Figure 2C). On the opposite side, the α1-α2 pair includes the binding site with the receptor *Hs*Pex5, as well as for *Hs*Pex19 (Neufeld et al. 2009; Neuhaus et al. 2014) (Figure 2C).

The site exhibits two hydrophobic pockets, which are separated by two aromatic residues, Phe35 and Phe52 (Neufeld et al. 2009; Neuhaus et al. 2014) (Figure 2Cii). The conserved aromatic residues in the WxxxF/Y motif of *Hs*Pex5, Trp118 and Phe122, directly fit into the hydrophobic pockets of Pex14(N) (Neufeld et al. 2009; Su et al. 2009) (Figure 2Ciii). Furthermore, complementary charges between positively charged residues surrounding the hydrophobic pockets of *Hs*Pex14p(N) and negative patches of the *Hs*Pex5 ligand helix, including the WxxxF/Y motif, stabilize the interface. An additional *Hs*Pex14 binding motif (LxxxF), binding with lower affinity to the N-terminal than the previous identified WxxxF/Y has been found in Pex5 (Neuhaus et al. 2014) (Figurer 2Civ). Albeit this interaction competes with binding of *Hs*Pex19 (Neufeld et al. 2009) (Figurer 2Cv), β-tubulin (Reuter et al. 2021) and even the membrane surface (Gaussmann et al. 2021) (see below), Pex5 shows the highest affinity and therefore displaces the other bound proteins (Neufeld et al. 2009).

The minimal region of *Hs*Pex14 required to recover impaired protein import in mammalian cells includes residues 21–260 (Itoh and Fujiki 2006), which further confirms that the conserved N-terminal domain, the TM helix and the coiled-coil domain are essential for Pex14 function. Little is known however about the functional role of the unstructured C-terminal domain. Phosphorylation in specific C-terminal sites of Pex14 was shown to affect import efficiency of Catalase (Okumoto et al. 2020; Yamashita et al. 2020) and Cit2p (Schummer et al. 2020) in mammalian and yeast cells, respectively (Niederhoff et al. 2005). The ORF14 protein of the human pathogenic virus SARS-CoV-2 has been shown to target the C-terminal domain of Pex14, resulting in an import defect and mislocalisation of matrix proteins to the cytosol in SARS-CoV-2 infected cells (Knoblach et al. 2021).

Recent cryoEM and cross-linking MS data suggest that *Sc*Pex14 further oligomerizes to form characteristic homotrimers, resembling flexible rod-like particles (Lill et al. 2020) (Figure 3C). The elongated rods are formed by the two coiled-coil-domains, being perpendicular to the membrane (Lill et al. 2020). In addition to the strong interactions between the coiled-coil domains, further interactions are expected between the conserved AXXXA and GXXXG motifs of the TM domains, which are common helical interaction motifs in membrane proteins (Kleiger et al. 2002). Their ability to promote oligomerization has been indeed verified in the mammalian Pex14 isoform (Itoh and Fujiki 2006).

It is well established that the flexible unstructured C-terminus (and thus the rods formed by the coiled-coil domains) are directly facing the cytosol, but the data on the membrane topology of the N-terminal domain of Pex14(N) are rather contradictory. This is however a very important point, since this multi-purpose protein-interaction platform is crucial for peroxisomal function (Gaussmann et al. 2021; Neufeld et al. 2009; Reuter et al. 2021; Su et al. 2009).

We would like to emphasize here, that the C-terminus of *Sc*Pex14 includes an exclusive second site of interaction with the PTS receptors (Niederhoff et al. 2005). Most importantly, deletion of the N-terminal PTS binding site of *Sc*Pex14 reduces PTS1 matrix protein import, whereas the truncation of the C-terminal binding site completely blocks it (Niederhoff et al. 2005). It should also be noted, that the

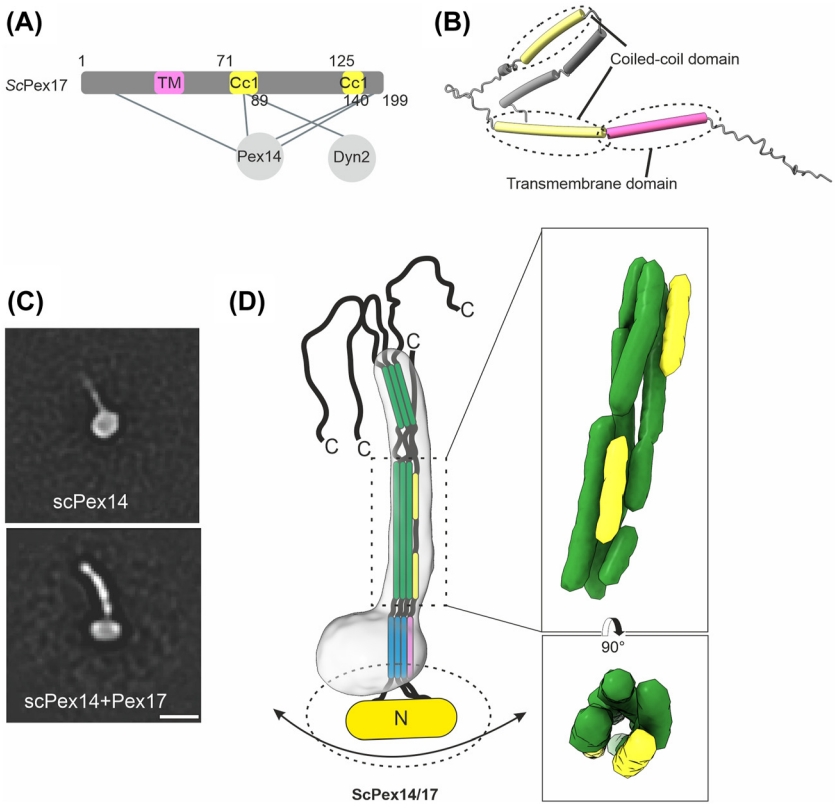

Figure 3: Pex17 stabilizes the peroxisomal docking complex.
Schematic (A) and alphafold 2 predicted secondary (B) structure (AF-P40155-F1-model_v2) of ScPex17 with the predicted transmembrane (TM, pink) and coiled-coil (Cc, yellow) domains, unstructured regions (grey). Mapped interaction sites of ScPex17 with additional components of the docking complex are displayed. (C) 2D-class averages of recombinant ScPex14 (C) and ScPex14/ScPex17 complex reconstituted into MSP1D1Δ4 nanodiscs (taken from (Lill et al. 2020)). (D) Schematic cartoon of the ScPex14/ScPex17 (green/yellow) complex arrangement in a 3:1 stoichiometry fitted into the cryoEM map of ScPex14/ScPex17 reconstituted in the MSP1D1Δ4 lipid nanodisc (Lill et al. 2020). Site and top view of simulated volume of the helical bundle assembly forming the ScPex14/ScPex17 rod. The volume was computed from polyalanine α-helices of coiled-coil domain 1 of Pex14 and Pex17, as identified in the original cryoEM map. (EMDB-12047) (Lill et al. 2020).

yeast PTS2-receptor *Sc*Pex7 binds exclusively to the C-terminus of *Sc*Pex14 (Niederhoff et al. 2005). This suggests the C-terminal sites (facing the cytosol) as the primary docking sites for the PTS receptors in yeast and a different role for the binding-site in the N-terminal domain.

This is in contrast to the literature data for *Hs*Pex14, which is suggested to exclusively bind PTS receptors via its N-terminal domain (Gouveia et al. 2000; Itoh and Fujiki 2006; Schliebs et al. 1999). This might also support the notion that several aspects of the mechanism of receptor recognition and subsequent cargo translocation might differ in detail in human and yeast.

The yeast Pex14/Pex17 complex

*Sc*Pex17 is considered as an additional core component of the docking complex in yeast, but so far a homologue has not been yet detected in higher eukaryotes (Smith et al. 1997). *Sc*Pex17 is a helical peroxisomal membrane protein with a putative TMD at residue 35 to 55 and two coiled-coil domains at residue 71 to 89 and 125 to 140 exposing into the cytosol (Chan et al. 2016; Lill et al. 2020) (Figure 3A). The TMD and each coiled-coil domain have been predicted by alphafold2 (AF-P40155-F1-model_v2) as α-helices separated by flexible linker of different length which is in line with previous predicted domain organization showing a linear organization (Figure 3B). *Sc*Pex17 was shown to highly increase the efficiency of receptor docking at the peroxisomal membrane, both for the PTS1 and PTS2 import pathways (Chan et al. 2016; Girzalsky et al. 2006). Recent cryoEM studies on *Sc*Pex14/Pex17 indicate that *Sc*Pex17-binding further stabilizes the rod-shaped homotrimers of *Sc*Pex14 (Lill et al. 2020) (Figure 3C). Along this line, a recent cryoEM structure of the *Sc*Pex14/*Sc*Pex17 complex reconstituted in lipid nanodiscs, in combination with cross-linking-, native-MS and size-exclusion chromatography combined with multi angle light scattering (SEC-MALS) data, revealed a 3:1 stoichiometric assembly and a parallel arrangement of *Sc*Pex14 and *Sc*Pex17p along their complete sequence (Lill et al. 2020). A single copy of *Sc*Pex17 is thereby running parallel to the elongated rod, that is mainly formed by three copies of the predicted coiled-coil domains of *Sc*Pex14 (Lill et al. 2020) (Figure 3D). Thus, the coiled-coil domain of *Sc*Pex17 is closely associated with this characteristic homotrimeric parallel helix arrangement, resulting in an intriguing helix bundle with pseudo four-fold symmetry (Lill et al. 2020) (Figure 3D). Lill et al. (2020) have identified a total of 13 residue pairs over both coiled-coil domains using cross-linking MS in combination with quantitative MS involved in these interactions. The bundle does not have a typical

rigid coiled-coil structure but contains multiple kinks and weak connections that could allow for a higher degree of flexibility and the expected large conformational changes in binding of cargo-loaded PTS receptors to *Sc*Pex14. The TM domain (a total of 4 TM helices) of *Sc*Pex14/*Sc*Pex17 is not resolved in the cryoEM density.

The C-terminal density beyond the coiled-coil domains also did not reach interpretable resolution, which further confirms a higher degree of flexibility, matching the alphafold prediction (Figure 2B). Interestingly, the flexible 120 aa unstructured C-termini of *Sc*Pex14, including the primary PTS receptor binding sites in yeast, do not exhibit homo-multimeric linkages (Chan et al. 2016; Lill et al. 2020). It is tempting to speculate that *Sc*Pex14/*Sc*Pex17 functions similar to a fishing rod. The coiled-coil region may be thought of as the "blank", the C-terminal elongated peptides as the "line", and the PTS receptor binding site as the "bait and hook" (Figure 3D). Together, these components can "fish" for cargo-loaded receptors and bring them in close vicinity to the peroxisomal membrane for further translocation events (Lill et al. 2020).

Several 2D cryoEM averages of *Sc*Pex14/*Sc*Pex17 in Lill et al. (2020) show the N-terminal globular domain of Pex14 as a flexible density below the nanodisc. This is consistent with the alphafold prediction showing the N-terminal globular domain connected to the TM domain via a long flexible linker (Figure 2B). Immunogold labeling of the N-terminus of *Sc*Pex14 further confirms that both termini are separated by the lipid bilayer in the *Sc*Pex14/*Sc*Pex17 complex (Lill et al. 2020). This agrees with the N_{in}-C_{out} topology proposed for the *Hs*Pex14 complex based on protease protection assays of *Hs*Pex14 complexes reconstituted in proteoliposomes (Barros-Barbosa et al. 2019). However, the topology of Pex14 is expected to be far more dynamic which will be addressed later within this review.

The *Sc*Pex14/*Sc*Pex17 complex is to our knowledge the minimal peroxisomal docking complex in yeast (Meinecke et al. 2010). However, high molecular mass complexes incl. Pex14, Pex17, Pex13 and the components of the export machinery, Pex10 and Pex12, were isolated from peroxisomal membranes upon affinity purification of Pex5, indicating that higher order molecular weight complexes must be assembled for efficient import (Chan et al. 2016; Meinecke et al. 2010). The dynein light chain protein Dyn2 is an additional component of the docking complex exclusively in yeast (Chang et al. 2013, 2016) and was shown to associate with the coiled-coil domains of Pex14 and Pex17 in a Pex17-dependent manner. In addition, it has also been shown to interact with the SH3-binding motif of *Sc*Pex13 (Chan et al. 2016). Dyn2 is proposed to act as a molecular glue, supporting formation and/or stabilization of large molecular assemblies of the importomer, similar to its role in stabilizing the Nup82-Nsp1-Nup159 complexes of cytoplasmic pore filaments of the nuclear pore complex (Chang et al. 2013; Stelter et al. 2007). Indeed, absence of Dyn2 results in the decrease of the higher order complexes of the docking/translocation machinery. It is however not yet clear whether physical binding between HsPex14 and HsDyn2 also occurs in human cells.

Pex13 - the other key component of the docking complex

Yeast and mammals share Pex13 as an additional membrane component of the peroxisomal docking complex crucially involved in the PTS1 and PTS2 import pathways (Elgersma et al. 1996; Erdmann and Blobel 1996; Gould et al. 1996). The ~42–44 kDa Pex13 is composed of two structural conserved regions: several putative transmembrane domains flanking a Pex14 binding site (Schell-Steven et al. 2005) and a highly important SH3 domain (Gould et al. 1996) (Figure 4A). The structure of the globular ScPex13 Src homology 3 (SH3) domain has been solved and analyzed using X-ray crystallography and NMR spectroscopy (Douangamath et al. 2002; Pires et al. 2003; Williams and Distel 2006). The overall β-barrel fold exposes a patch of hydrophobic residues forming a pocket suitable for the PXXP motif of Pex14(N) from yeast (Pires et al. 2003) and human (Douangamath et al. 2002) (Figure 4B). Furthermore, the opposite site exposes an additional hydrophobic binding site with specificity for the N-terminal WXXXF/Y motif of Pex5 (Bottger et al. 2000; Douangamath et al. 2002; Pires et al. 2003) (Figure 4B). Therefore, the SH3 domain of Pex13 is considered a critically switch in the import process and its absence or defect results in growth defect on methanol in yeast (Gould et al. 1996) or peroxisome deficiency in human (Krause et al. 2013).

Indeed, Pex5 was shown to bind to either Pex14 or Pex13 in a cargo-dependent manner in mammals, with higher affinity for Pex14 when cargo is bound and for Pex13 when cargo is not bound (Otera et al. 2002; Urquhart et al. 2000). Furthermore, the major fraction of *Sc*Pex13 does not co-purify together with ScPex14/17 (Agne et al. 2003), cargo-free Pex5 induces the disassembly of Pex14-homo-oligomers (Itoh and Fujiki 2006) and Pex14 and Pex5 were shown to compete for binding to the SH3 domain of Pex13 (Urquhart et al. 2000). There is thus a dynamic interplay between Pex5, Pex14 and Pex13, involving numerous interactions, which apparently however largely depend on the respective stage of the cargo-docking and -unloading

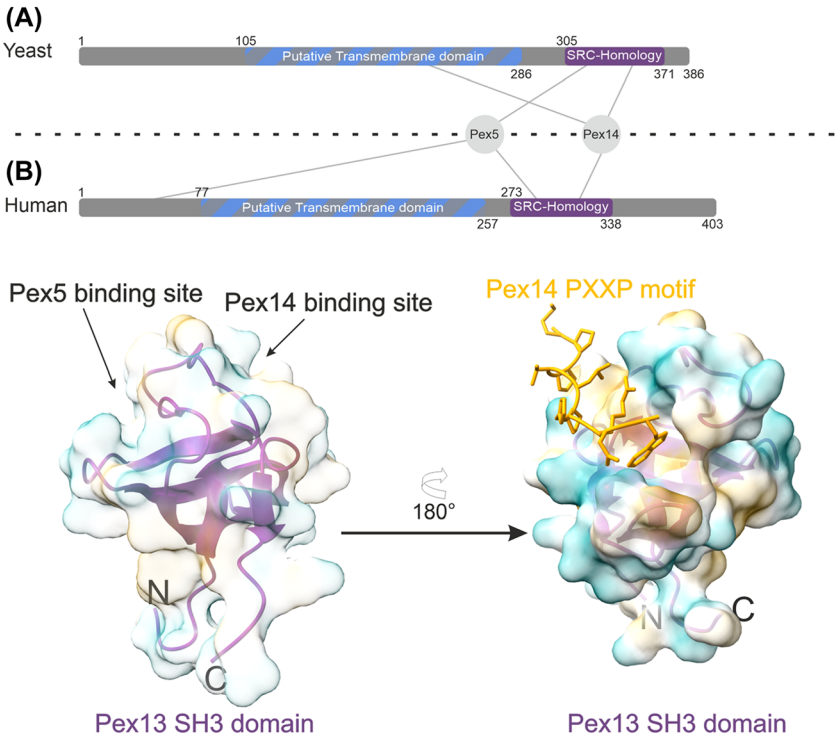

Figure 4: Pex13 interacting with Pex5 and Pex14.
(A) Schematic structure of the yeast and human Pex13 protein. Unstructured regions are displayed grey. The putative transmembrane region (blue striped) was predicted with TMHMM 2.0 (Krogh et al. 2001; Sonnhammer et al. 1998). The prediction did not allow to interpret whether the four identified transmembrane domains are full spanning. Therefore, we avoid any assumptions about the actual transmembrane topology. The Src homology 3 (SH3) domain is displayed purple.
(B) Structure of the Pex13 SH3 domain (PDB: 1N5Z). The color of the surface represents the level of hydrophobicity (yellow: Hydrophobic, blue: hydrophilic). The SH3 domain exposes two opposite binding sites for the Pex5 WXXX(F/Y) and Pex14 PXXP motifs. The actual binding of the Pex14 PXXP motif to the Pex13 SH3 domain is shown.

events. Based on the assumption, that the described interactions properties reflect the situation *in vivo*, it has been postulated that cargo-loaded Pex5 initially binds with high affinity to Pex14. Binding to Pex13, might occur later, during or after cargo release, mediating thus the shuttling of the receptor back to the cytosol (Rayapuram and Subramani 2006; Urquhart et al. 2000).

The fact that Pex5 contains a single LVAEF site and multiple sequential and linear WXXX(F/Y) binding motifs to Pex14, has led to the suggestion that Pex14(N) might first "dock" to LVAEF and then step-by-step, "slide" along the respective sequence of cargo-loaded Pex5 (Neuhaus et al. 2014; Schliebs et al. 1999). These events might allow transfer of the Pex14(N) towards the SH3 of Pex13 and of the cargo towards the peroxisomal matrix. This might induce pore assembly and finally cargo release once the two WXXX(F/Y) downstream motifs six and seven are reached (Freitas et al. 2011; Neuhaus et al. 2014). Pex13 binds to WXXX(F/Y) motifs 2–4 of Pex5, and according to the "sliding"-model, might thus be crucial in pore-assembly or disassembly and cargo release (Neuhaus et al. 2014). This might also partially explain the controversy on the membrane topology of the Pex13(SH3) (Barros-Barbosa et al. 2019), but the required biochemical and structural data on these events have remained scarce.

Conclusions and future perspectives

Pex14 is a central membrane protein in peroxisome biology. Binding of the cargo-loaded receptors to Pex14 triggers a plethora of interactions and the formation of higher-order complexes. These are required for the formation of the translocation channel and subsequent receptor recycling.

Furthermore, *Hs*Pex14 anchors peroxisomes to microtubules with nanomolar affinity (Bharti et al. 2011; Reuter et al. 2021) playing an important regulatory role in peroxisome mobility (Neuhaus et al. 2016). *Sc*Pex14 binds on the other hand strongly to Dyn2 (Chang et al. 2013, 2016), but this has been correlated rather with a direct role in the importomer assembly and not in peroxisome motility (Chang et al. 2013). More recently, a competitive correlation between matrix protein import and anchoring to microtubules has been suggested, as the PTS1 receptor *Hs*Pex5 prevents *Hs*Pex14 binding to β-tubulin (Reuter et al. 2021).

Recombinantly expressed *Sc*Pex14/*Sc*Pex17 was recently reconstituted in liposomes and subsequently visualized by cryo electron tomography: Rod-shaped filamentous

*Sc*Pex14/17 particles were shown to isotropically decorate the surface of liposomes without forming higher-order assemblies or affecting membrane curvature (Lill et al. 2020). For such a versatile binding platform for cargo-shuttling receptors and microtubule tethering, a membrane-bound, rod-shaped filamentous structure would certainly present advantages. It is tempting to speculate that peroxisomes might be decorated in a similar "sea urchin"-like manner by large numbers of flexible *Sc*Pex14/*Sc*Pex17 filament particles, given that Pex14 is the most abundant peroxisomal membrane protein and native *Sc*Pex14/17 complexes solubilized from whole cell membranes, do exhibit this characteristic elongated architecture (Lill et al. 2020). However, the docking complex has not yet been visualized directly at the peroxisomal membrane. Despite recent developments in cryo-EM, the elongated and highly flexible structure of Pex14 poses a major challenge in this direction. Moreover, the addition of important components of the docking complex, such as Pex13 or Dyn2 in yeast, could lead to transient complexes with completely different architectures. Nevertheless, the filamentous structure of the yeast Pex14 complex, with its primary PTS receptor binding site at the flexible C-terminal tail facing the cytosol (Niederhoff et al. 2005) and the second N-terminal receptor binding site on the opposite site of the membrane, renders a two-step model of receptor docking attractive:

(a) in a first step, the C-terminus of *Sc*Pex14 is the actual docking site for the receptor *Sc*Pex5 (Niederhoff et al. 2005)
(b) This event triggers formation of a pore by *Sc*Pex5p (Erdmann and Schliebs 2005; Meinecke et al. 2010). Alternatively, the pore is formed by the components of the docking complex (Dias et al. 2017).
(c) interaction between cargo-loaded *Sc*Pex5 and the matrix-facing N-terminal domain of *Sc*Pex14 would thus occur in a second step either after membrane insertion of *Sc*Pex5 (Lill et al. 2020; Niederhoff et al. 2005) or its passage through a central cavity formed by the docking complex (Dias et al. 2017). This interaction may be important for the formation of the translocation channel, its stabilization (Lill et al. 2020; Niederhoff et al. 2005) and/or cargo release.

In this sense, several studies are in agreement on the N_{in}-C_{out} topology of Pex14 in different organisms (Barros-Barbosa et al. 2019; Lill et al. 2020; Oliveira et al. 2002), whereas a plethora of studies rather suggests the involvement of Pex5 into pore formation events. Pex14 has been shown to form a stable complex with membrane-bound Pex5, which in turn exhibits properties of an integral membrane protein (Gouveia et al. 2000; Kerssen et al. 2006; Urquhart et al. 2000). Binding of *Hs*Pex5 shows also significant affinity to the membrane and docking of the *Hs*Pex5 to *Hs*Pex14 at the membrane does not alter the affinity between both proteins (Gaussmann et al. 2021).

However, with respect to mammalian Pex14, there are the following important arguments against such a two-step mechanism: If the N-terminal domain is the only structural region of mammalian Pex14 containing a receptor binding site, a N_{in}-C_{out} topology would be inconsistent with its main function of “fishing” cytosolic receptors ferrying cargos (Oliveira et al. 2002; Shimizu et al. 1999). In addition, Shimizu et al. (1999) suggested a N_{out}-C_{out} topology based on epitope labeling of the termini (Shimizu et al. 1999). Most importantly, a N_{in}-C_{out} topology is also inconsistent with the high-affinity binding of *Hs*Pex14 to β-tubulin required for peroxisome mobility, because the β-tubulin-binding site of *Hs*Pex14 is localized in the N-terminal domain (Bharti et al. 2011; Reuter et al. 2021). A simple explanation might be that several aspects of the underlying mechanisms might differ in detail between yeast and mammalian Pex14. For example, *Hs*Pex14 does not include the second predicted coiled-coil domain and is not further stabilized by Pex17. However, it is rather interesting to conclude, given the overall structural conservation of Pex14, that such conflicting results might rather represent mechanistic snapshots of a very dynamic complex.

This view would agree with the recent exciting finding that mitotic phosphorylation of *Hs*Pex14 induces drastic conformational changes that result in the protection of Pex14(N) from proteolytic digestion, indicating thus in this case a clear topological change of the N-terminal domain of *Hs*Pex14 (Yamashita et al. 2020). Furthermore, interaction with other membrane peroxins of the importomer machinery and assembly of higher order complexes, might also affect the topology of the N-terminal domain. For example, the Pex14(N) might shift from the cytosol into the peroxisomal matrix upon Pex5 docking, following the expected drastic conformational changes of cargo-loaded Pex5 and its insertion into the membrane. Such conformational changes would be also in accordance with the “sliding” model (Neuhaus et al. 2014), with the N-terminal domain of Pex14 sliding and “scanning” the sequential WXXX(F/Y) binding-motifs of Pex5, with each dislocation triggering a step of the process (docking → membrane insertion → pore assembly → cargo release → pore disassembly). The precise role of the other core component of the docking complex Pex13, in this process, remains unclear.

One of most exciting challenges towards understanding this complex series of events triggered upon receptor-

cargo docking at the peroxisomal membrane, remains thus the detailed structural investigation of the importomer and exportomer machineries and their individual components.

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Note added in proof: During the review of this article, we became aware of a recent study by Skowyra and Rapoport (2022) supporting the latter model, in which Pex5 rather crosses the membrane and moves into the peroxisomal lumen to release its cargo. The mechanistic details and precise role of the docking complex and Pex14 in this process remain enigmatic.

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