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Minireview

Protein translocation into peroxisomes by ring-shaped import receptors

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Abstract **Folded and functional proteins destined for translocation from the cytosol into the peroxisomal matrix are recognized by two different peroxisomal import receptors, Pex5p and Pex7p. Both cargo-loaded receptors dock on the same translocon components, followed by cargo release and receptor recycling, as part of the complete translocation process. Recent structural and functional evidence on the Pex5p receptor has provided insight on the molecular requirements of specific cargo recognition, while the remaining processes still remain largely elusive. Comparison of experimental structures of Pex5p and a structural model of Pex7p reveal that both receptors are built by ring-like arrangements with cargo binding sites, central to the respective structures. Although, molecular insight into the complete peroxisomal translocon still remains to be determined, emerging data allow to deduce common molecular principles that may hold for other translocation systems as well.**

Keywords: Peroxisomal translocation; Import receptor; Tetratricopeptide repeat motif; Solenoid fold; Cargo recognition; Transient translocon

1. Introduction

Peroxisomes are inducible multi-purpose organelles, sequestering metabolic pathways that would otherwise be toxic if they were allowed to occur in the cytosol. While the precise set of biochemical reactions occurring within peroxisomes differs amongst species, tissues, and environmental conditions, the decomposition of reactive hydrogen peroxide by catalase is an ubiquitous feature of peroxisomal function. In contrast to some other cell organelles, such as mitochondria and chloroplasts, peroxisomes do not contain discrete genomes and are thus entirely dependent on external biosynthesis of the proteins required for their formation and function. Therefore, peroxisomes require specific mechanisms for protein translocation.

The translocon required for the import of peroxisomal matrix proteins appears to be composed of about a dozen *peroxins*, which collectively have been termed *importomer* [1,15,46]. Several protein components involved in importomer formation show dynamic localization patterns, indicating active and dynamic participation in transport processes across the peroxisomal membrane. The majority of proteins destined for translocation are recognized by the cytosolic import receptor Pex5p *via* a C-terminal peroxisomal targeting signal type 1 (PTS1) motif [6,56]. While the C-terminal part of the receptor, which consists of an array of tetratricopeptide repeat (TPR) motifs, is sufficient for canonical PTS1 recognition, there is increasing evidence for the involvement of the same receptor in PTS1-independent translocation of peroxisomal enzymes with either additional or exclusive binding sites within the N-terminal part of the receptor [58].

A smaller number of peroxisomal matrix proteins contains an alternative peroxisomal targeting signal type 2 (PTS2) motif close to the N-terminus. These enzymes are recognized by a second cytosolic receptor, Pex7p [34]. In contrast to Pex5p, which is capable of target recognition and docking at the peroxisomal membrane, Pex7p requires additional protein components for peroxisome targeting, thus functioning as a PTS2 co-receptor [48]. Although, these co-receptors differ amongst species, they share a characteristic N-terminal segment, a number of WxxxF/Y sequence motifs, and a Pex7p binding region. It is noteworthy that evidence from sequence analysis indicates that the PTS1 receptor Pex5p of plants, animals, protista, and several fungi on its own contains a Pex7p binding region, suggesting an additional role for Pex5p as a Pex7p co-receptor [48]. In mammalian Pex5p, the Pex7p binding site has been mapped to a 37-residue insert that is only present in the long, alternative splice variant of the receptor, Pex5pL [12,39]. Because of the function of Pex5p (or structurally-related peroxins in yeast species) as a Pex7p co-receptor, it is not surprising that both protein translocation systems bind to the same two docking components at the peroxisomal membrane, Pex14p and/or Pex13p [4,22,61]. Knowledge of the precise mechanisms for cargo translocation and cargo release remains fragmentary and is subject to an ongoing debate [15,32,46].

The availability of the molecular structures of the participating protein components and their resultant complexes would greatly help to identify the molecular mechanisms responsible for protein translocation across the peroxisomal membrane. However, the *in vitro* instability of most peroxins and the transient nature of many known interactions have made structural characterization a difficult task. Nonetheless, we have recently

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been able to elucidate the structure of a protein cargo complex of the human Pex5p receptor [52]. In contrast, experimental structural data of the Pex7p receptor are still lacking. In this review, we summarize the present knowledge on the molecular function of these two peroxisomal import receptors that has been garnered either from experimental structural data or from computational predictions.

1.1. Molecular basis of PTS1 cargo recognition by the Pex5p receptor

The cargo binding (CB) domain of the Pex5p receptor is formed by a sequential arrangement of seven tetratricopeptide repeat (TPR) motifs, followed by a C-terminal bundle of three helices [20,52] (Fig. 1A). TPR motifs comprise 34 residues with a characteristic sequence signature, each forming a two-helical bundle [10]. They are found in a broad range of proteins amongst both prokaryotes and eukaryotes and are generally involved in protein–protein interactions. TPR motifs are frequently arranged in repetitive arrays, forming superhelical solenoid structures with about eight TPR motifs per turn and a helical pitch of about 70 Å [25,27,30]. However, in the CB domain of the Pex5p receptor, this pattern is interrupted by a non-canonical conformation of the fourth TPR motif that is only partly ordered in the available Pex5p receptor structures [20,52,53]. The peculiar conformation of TPR4 separates the remaining TPR motifs into two triplets, TPR1–TPR3 and TRP5–TPR7, each with arch-like arrangements. The overall architecture of all seven TPR motifs is that of an ellipsoidal shade (Fig. 1A). In the cargo-bound conformation of the Pex5p receptor, the seven TPR motifs form a closed ring arrangement. A side view of the CB domain structure of Pex5p reveals that the overall arrangement of the sevenfold repeated TPR array is bent, leading to two non-equivalent disk surfaces, with an inner, convex face and an outer, concave face (Fig. 1A). A remarkable feature of the Pex5p CB domain is the presence of a central tunnel through the disk shaped structure (Fig. 2C).

In the presence of a pentapeptide with the C-terminal PTS1 and a complete PTS1-containing protein, sterol carrier protein 2 (SCP2), the Pex5p receptor structures consistently show how the PTS1 motif binds into the central tunnel, about half-way through the disk-like structure of the CB domain of the receptor (Fig. 2B) [20,52]. Most of the specific PTS1-receptor interactions are generated by four conserved asparagine residues from the CB domain, which are located in TPR repeats 3, 6, and 7 [20,28,52]. Of these, two form direct hydrogen bond interactions with the C-terminal carboxylate group of the PTS1 motif. The C-terminal leucine side chain of the PTS1 cargo motif is entirely buried within the receptor (Fig. 2A,C). However, none of the other side chains of the C-terminal PTS1 tripeptide are involved in specific interactions with the CB domain of the receptor, thus supporting the formation of the loose C-terminal tripeptide consensus found amongst known peroxisomal proteins [6]. In contrast to the interactions of the C-terminal PTS1 tripeptide motif that are consistent in both available Pex5p–PTS1 complex structures, the conformation of the residues preceding the C-terminal tripeptide motif are different in the structures of the receptor-PTS1 peptide

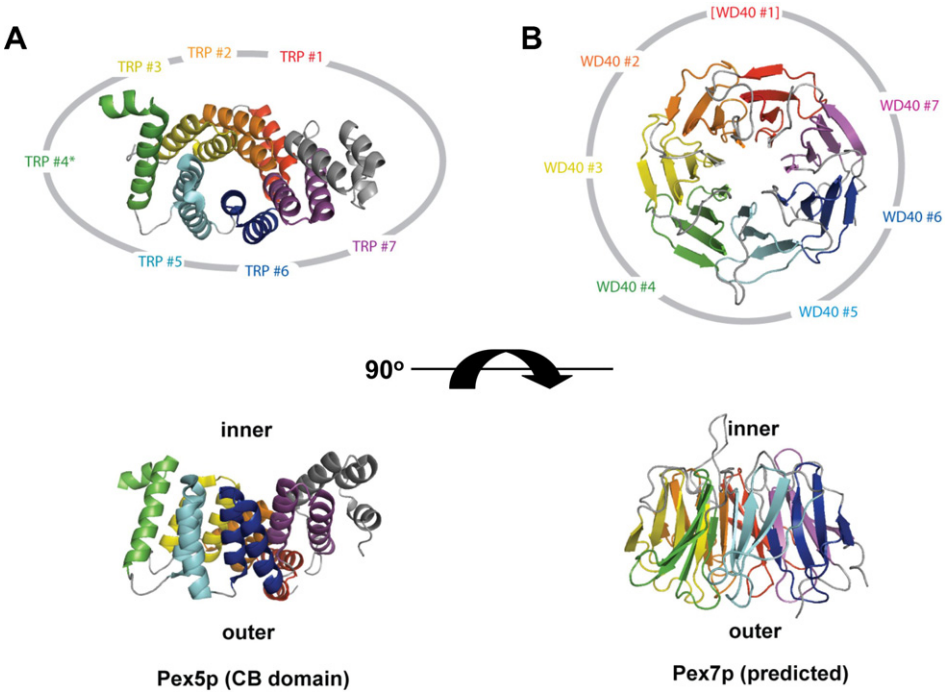

Fig. 1. Comparison of the CB domains of Pex5p and Pex7p. (A) Structure of the CB domain of the human Pex5p receptor. The seven TPR repeats are numbered counterclockwise and colored in rainbow colors. The C-terminal helical bundle, which is not part of the TPR array, is colored in grey. (B) Comparative protein structure model of Pex7p based on a seven-bladed WD40 propeller template (PDB code: 2H9N). The colors of the seven blades are numbered and in rainbow colors. Although the complete model is shown, we cannot reliably detect the first WD40 blade motif in the Pex7p sequence. Therefore, the number of the corresponding blade is indicated in square brackets. The repeat domains in both structures form ring-like arrangements, which are either elliptical or circular. The side view of both structures (lower panel) demonstrates that both resultant disk-like shapes are bent, creating an inner, concave and an outer, convex surface. The two surfaces are labeled "inner" and "outer".

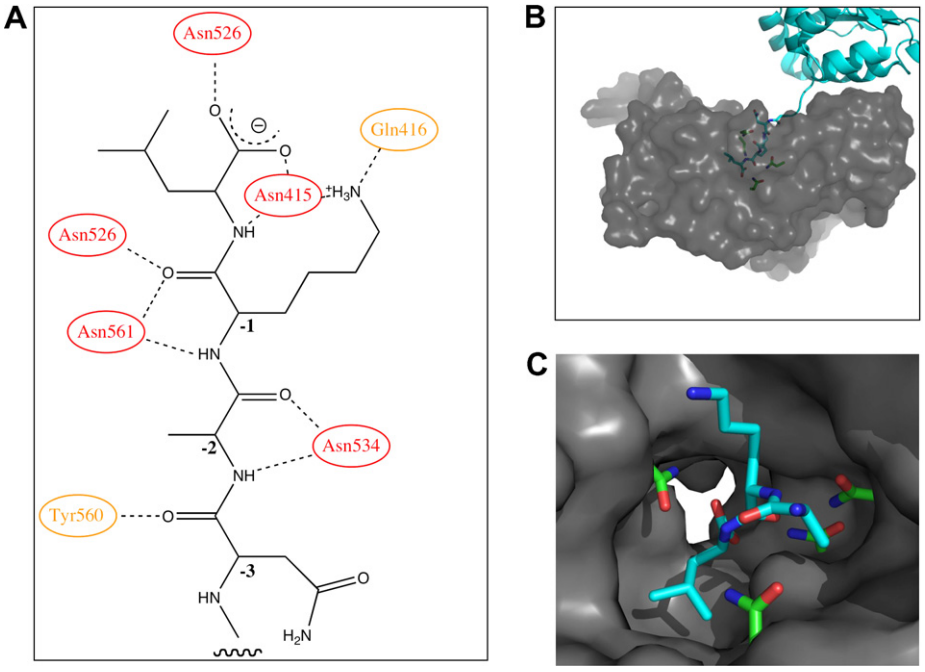

Fig. 2. PTS1 cargo recognition by the Pex5p receptor. (A) Specific interactions of the C-terminal PTS1 peptide with the CB domain of Pex5p, as determined by the crystal structures of Pex5p complexes with a PTS1 peptide (PDB code: 1FCH) and the PTS1-containing peroxisomal matrix protein SCP2 (PDB code: 2C0L). All indicated interactions are consistently found in both structures. The residue positions of the C-terminal PTS1 peptide are numbered in reverse order, in which the C-terminus has been associated with position 0. Pex5p residues involved in specific side and main chain interactions are schematically displayed in red and orange colors, respectively. (B) Mixed surface/ribbon representation of the Pex5p/SCP2 complex in grey and atom type colors (carbon, cyan; oxygen, red; nitrogen, and blue), respectively. The C-terminal PTS1 motif penetrates the central tunnel of Pex5p about half way from the inner, convex surface of the CB domain. (C) View of the Pex5p bound C-terminal PTS1 peptide along the central tunnel of the receptor CB domain, colors as in (B). In addition, the four conserved asparagines of the Pex5p CB domain sequence (cp. panel A) are shown in atom-type colored sticks (carbon, green; oxygen, red; nitrogen, and blue).

[20] and receptor/PTS1 protein (SCP2) [52] complexes. In the latter complex, these residues are not involved in any specific interactions with the receptor and form a bridge between the lipid binding domain of SCP2 and the PTS1 motif, which is entirely separated from the lipid binding domain in the receptor-bound form, without interfering with the function of SCP2 [52,55]. Indeed, available data from the Pex5p/SCP2 cargo complex indicate that the ability of those PTS1-preceding residues to associate with the remaining part of the cargo in the absence of the Pex5p receptor may be more important than their specific contribution to Pex5p recognition, thus limiting the predictive power of algorithms for PTS1 motif identification [14,21,42].

Analysis of the recent structural data of the cargo-bound and the apo-conformation of the Pex5p receptor has also revealed that cargo recognition leads to a conformational transition of the CB domain from a looser snail-like arrangement in the absence of cargo, to a ring-like arrangement in the presence of cargo [52,53]. Comparison with the previous Pex5p–PTS1 peptide complex [20] reveals that the presence of the C-terminal PTS1 motif is sufficient to trigger the observed conformational transition, locking Pex5p into the ring-like arrangement. Although the non-canonical TPR4 motif could be envisioned as the most plausible origin of conformational flexibility in Pex5p, the recent structural data have revealed that the ring opening of the sevenfold repeated TPR array is rather caused by a long loop C-terminal to the TPR7 motif (7C-loop) and a rotational motion centered around the TPR5/TPR6 motif tandem [52,53]. In the cargo-bound conformation, the 7C-loop connects TPR1 with TPR7, thus closing the seven-membered TPR ring, while in the snail-like apo-conformation of the receptor CB domain the loop is largely separated from the remaining TPR array. Interestingly, in the available Pex5p apo-structures, the 7C-loop interacts with a partially conserved residue motif N-terminal to the TPR array [52,53]. The importance of the 7C-loop in Pex5p receptor function is underlined by a well-characterized mutation (S600W) that leads to a severe clinical phenotype [50]. These conclusions are further supported by in vivo translocation assays, using several Pex5p variants with single residue mutations in the 7C-loop [52].

1.1.1. Open questions about cargo recognition by the Pex5p receptor. There is increasing recent evidence that several PTS1-containing cargos can even be recognized by the Pex5p receptor in an PTS1 independent fashion, suggesting that there may be additional interactions that contribute to cargo recognition [24,56,58]. The structure of the Pex5p/SCP2 cargo complex indeed revealed a second ancillary binding site at the C-terminal helical bundle of the receptor, where several specific interactions with polar surface residues of the SCP2 cargo were observed [52]. However, although the in vitro binding affinity of the entire cargo is about fivefold higher than that of the C-terminal PTS1 peptide motif, a functional contribution of this secondary binding interface to peroxisomal PTS1-driven import in vivo has not been demonstrated (Schliebs et al. unpublished). Moreover, the C-terminal ancillary SCP2 cargo binding site is only partially conserved amongst the available Pex5p sequences [52]. As there are no other obvious conserved surface patches at the inner, convex surface of the CB domain

of the receptor, it remains difficult to predict whether there are different, ancillary CB domain binding sites for other cargos as well as to corroborate a principal molecular mechanism underlying receptor-PTS1 cargo recognition. Therefore, in order to fully unravel the complete arsenal of possible structural dynamics of the receptor upon cargo recognition, experimental structures of additional receptor/cargo complexes are required, beyond the only available structure to date, that of the Pex5p/SCP2 complex. Both, the detection of additional cargo binding sites and the characterization of possibly unknown additional conformational changes of the receptor upon cargo binding, could provide insight into the yet unknown molecular mechanism of PTS1 cargo sorting prior to translocation.

Indeed, present structural and functional analyses have been seriously hampered by the inability of many PTS1 cargos to passively bind to the CB domain of the Pex5p receptor in vitro [17] (Fodor and Wilmanns, unpublished). Comparison of the available SCP2 structures in the presence or absence of the Pex5p receptor has revealed that the C-terminal PTS1 motif needs to disassemble from the remaining cargo in order to penetrate the central PTS1 binding site of Pex5p by more than 10 Å (Fig. 2B). The relative loose arrangement of the C-terminal tail, observed in previous SCP2 apo-structures [7,19], may provide a molecular rationale as to why receptor binding to purified SCP2 is not impeded. However, association of the PTS1-containing C-terminal tail with the functional domains may be tighter in other PTS1 cargos, thus rendering more difficult any conformational adjustment of the PTS1 C-terminus that is required for Pex5p receptor binding. For instance, available structural data of peroxiredoxin 5 could explain why, in our experience, it is not possible to bind in vitro purified enzyme to the Pex5p receptor (Fodor and Wilmanns, unpublished). Comparison of the reduced and oxidized structures of peroxiredoxin 5 reveals conformational changes of the PTS1-containing C-terminal sequence region [11,16], which could serve as a determinant for Pex5p receptor recognition.

It is also noteworthy that most of the available structural data have been obtained from experiments using only the C-terminal PTS1 cargo binding part of the receptor. Although various investigations have demonstrated functional autonomy of the C-terminal PTS1 cargo binding part of the receptor, recent reports on bipartite recognition of some PTS1 cargos by additional binding sites, mapped onto the N-terminal part of the receptor, suggest that there could be more cross-communication and dependence amongst different parts of the receptor than initially anticipated [56,58]. Such cross-communication is also supported by a recent hypothesis that recognition of the Pex5p receptor by the docking complex is mediated by cargo loading [22].

While some of the structural parameters required for cargo recognition by Pex5p have been established, only little is known about the molecular mechanisms and structural dynamics involved in cargo release from the Pex5p receptor. Increasing evidence indicates that cargo release is a complex process that may involve several other peroxins, such as the membrane-bound RING finger-containing peroxins (Pex2p, Pex10p, and Pex12p). In addition, in several yeast species, the function attributed to Pex8p is one of an intraperoxisomal organizer of the docking and RING complexes [1,46]. It also remains to be determined whether cargo release and ubiquitination-dependent recycling of the Pex5p receptor occur sequentially or concomitantly during Pex5p receptor cargo translocation [45,57]. In conclusion, it still remains to be determined to what extent the observed structural dynamics of the Pex5p receptor, which have only been identified for SCP2 cargo docking, may also apply conversely for cargo release, or to what extent recycling of the apo-conformation of the receptor may follow a different route of conformational transitions.

Recent structural data on the apo-conformation of the Pex5p CB domain do indeed suggest potential overall disorder/order transitions of the sevenfold TPR array arrangement upon cargo recognition/release, beyond the established local conformational changes [52]. In the first published apo-structure, in two out of four copies of the Pex5p receptor, the electron density of the first TPR triplet (TPR1–TPR3) of Pex5p was too blurred to support a detailed structural interpretation. However, the presence of these folded TPR repeats could still be observed, indicating that they are indeed folded [52]. The crystallographic observations are consistent with previous biophysical data on Pex5p by small angle X-ray scattering and circular dichroism, which ruled out folding of the CB domain induced by cargo recognition [53,54]. Similar observations, indicating overall flexibility of folded structures, recently described as 'molecular spring', have been made for other proteins with large arrays of small α-helical domain modules involved in cell adhesion and nuclear transport processes [2,9,13]. Hence, intrinsic overall fold flexibility of the Pex5p receptor may also be an important, yet still largely unrecognized, parameter for cargo recognition/release.

Finally, the role of the flexible TPR4 domain, leading to two separate TPR triplets in Pex5p, will remain of particular interest in unraveling potential conformational dynamics. A recent structure of the mitochondrial translocation receptor Tom70 has indeed revealed that fragmented TPR arrays are not unique to Pex5p [62]. However, the two TPR arrays of Tom70, TPR1–TPR3 and TPR4–TPR11, form a head-to-head arrangement, in contrast to the ring/snail-like conformation in Pex5p, which can be described as a head-to-tail arrangement. Conversely, complete folding of the TPR4 repeat into a canonical TPR motif conformation, which has not yet been observed to date, may lead to a superhelical solenoid conformation of the CB domain of the receptor, consistent with observations from other proteins with extended, non-interrupted TPR arrays [25,27]. Conformational variability and unfolding, although unknown whether being of physiological relevance, has also been observed in recent structures of the first TPR triplet of the CB domain of the Pex5p receptor [31] and phosphatase 5 [8].

1.1.2. Cargo recognition by the Pex7p receptor. Pex7p is the ubiquitous receptor for peroxisomal proteins harboring a PTS2, which is defined by the consensus sequence RLxxxxx(H/Q)L near the N-terminus [34]. Like the PTS1 receptor, Pex7p shuttles between the cytosol and peroxisome during PTS2 cargo translocation [41]. In contrast to Pex5p, Pex7p requires interacting co-receptors to complete PTS2 cargo translocation [48]. Although in several yeast species, these co-receptors are capable of binding to PTS2 sequences [44,64], there is general agreement, however, that in most, if not all organisms, Pex7p provides the initial PTS2 recognition site of the heteromeric Pex7p/co-receptor complex in the cytosol.

In contrast to Pex5p, there are no experimental data available on the structure of Pex7p and potential conformational dynamics that may be associated with PTS2 cargo recognition. The

receptor was predicted to fold as a seven-bladed β-propeller domain [38,63], in which each 'blade' comprises the so-called WD repeat [35,51]. WD repeat motifs are formed by sequence patterns of 44–60 residues, which fold into a four-stranded antiparallel β-sheet domain. The first experimental structure of a seven-bladed WD40 β-propeller was that of the β-subunit of the heterotrimeric G protein complex [33,59]. In this structure, the outer β-strand of the C-terminal WD40 repeat is provided by the N-terminus of the same sequence, leading to a description of the WD40 repeat as one of a "d–a–b–c" β-sheet topology [51]. The sequence signature of the WD40 repeats includes a conserved GH motif in the d–a loop, an invariant aspartate in the b–c loop, and a conserved WD motif at the C-terminus of the β-strand c, after which the repeat was named. Although the Protein Data Bank contains nearly 100 structures with β-propeller arrangements, there are only 22 structures with WD40 repeat β-propellers, according to the classification scheme by SCOP [3]. In these structures, the WD40 repeats generally fold into seven-bladed propellers, although at least one eight-bladed WD40 repeat propeller has been found in the structure of an ubiquitin ligase [43].

Similar to the ring-like TPR array in the CB domain of Pex5p, these propellers form a disk-like shape, which is bent, thus creating an outer, concave surface and an inner, convex surface [51] (Fig. 1B). WD40 propellers, however, form circular structures as opposed to the ellipsoidal shapes found in the CB domain of Pex5p. Therefore, the individual domains ('blades') are at equal distances to each other, whereas the distribution of TPR repeats within the structure of the Pex5p CB domain is asymmetric (Fig. 1B).

The sequence of the human Pex7p comprises 323 residues. Available motif search methods reliably detect six WD40 repeats for Pex7p, covering most of its sequence except for the N-terminal part (residues 1–65) (Fig. 3). The C-terminal WD40 repeat in Pex7p appears to be truncated, reminiscent of previous structural findings in the WD40 propeller of the β-subunit of the heterotrimeric G protein [51]. Although a distinct sequence signature for the first WD40 repeat cannot be recognized, the entire Pex7p sequence, including the N-terminus, comprises a high β-sheet content, matching the secondary structural content of WD40 propellers (Fig. 3), thus supporting the completion of the seven-bladed WD40 β-propeller.

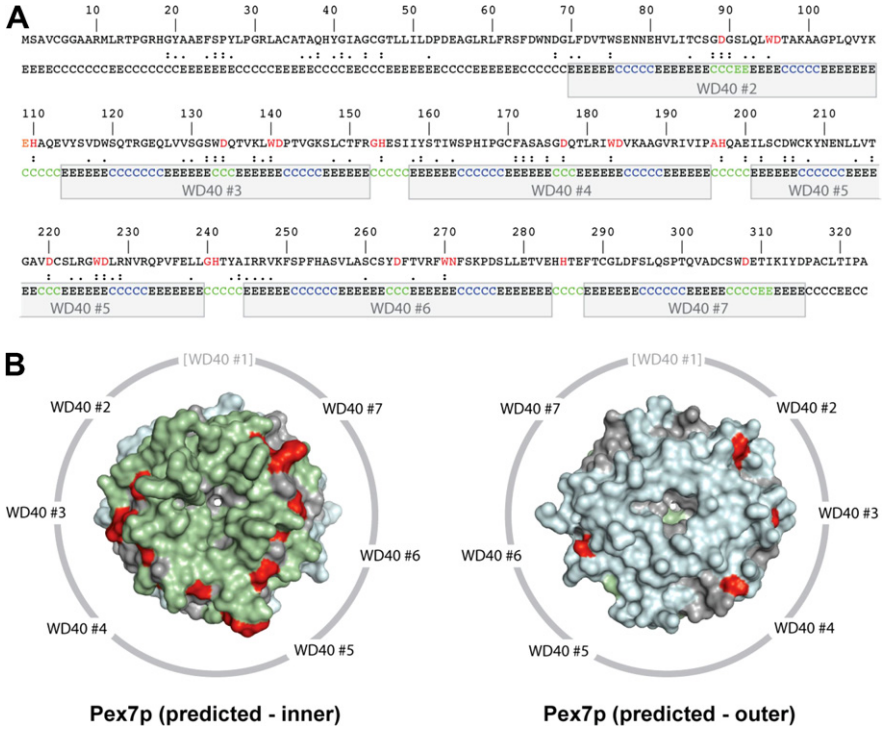

Fig. 3. Prediction of the structure and PTS2 binding site in Pex7p. (A) Sequence of the human Pex7p receptor (PEX7_HUMAN, O00628, upper line) threaded onto the structure of a related WD40 β-propeller, histone methyl-lysine recognition domain WDR5 (PDB code 2H9N), sharing 22% sequence identity. Residue numbers are indicated above the sequence. WD40 signature residues [51] are shown in red color. The central line indicates the positions of invariant (":") and highly conserved (".") residues, based on a multiple sequence alignment from 25 sequences, unambiguously identified as Pex7p (not shown). The lower line indicates the predicted secondary structure (E, β-sheet; C, coil) for Pex7p. The numbers and positions of the WD40 repeats have been taken from the structural template used for threading. WD40 domains generate an alternating pattern of loops, exposed to the inner surface and outer surface of the β-propeller fold. The corresponding loops are colored in green (inner surface) and blue (outer surface), respectively. (B) Structural model of Pex7p. Invariant and highly conserved residues, as defined above, are colored in red and orange, respectively. The inner and outer surface loops are colored in faint green and blue, respectively, matching the color coding in panel A. Comparison of the inner and outer surfaces of the Pex7p model demonstrates that most of the conserved residues are located at the inner surface, indicating its involvement in Pex7p function. Methods used for Pex7p structure prediction and modeling. The secondary structure of Pex7p was predicted by PSIPRED [26]. Both the mGenThreader [40] and 3D-JIGSAW [5] software packages identified PDB entry 2H9N as the most suitable template for the Pex7p target sequence. The MODELLER program [47] was used to optimize the alignment of the target (Pex7p) and model (2H9N) sequences and to build a structural model of Pex7p. The accuracy of the overall fold and localization of the most conserved elements was evaluated by DOPE [37,49] resulting in scores of −37 110.1 and −34 211.9 for the 2H9N template and the Pex7p model, respectively. The locations of predicted β-strands and those arising from threading the Pex7p sequence onto the structural template of WDR5 are virtually identical (not shown), thus cross-validating each type of prediction.

However, at this point, we cannot rule out an alternative model, in which the Pex7p β-propeller structure would be completed by the insertion of a WD40 module from a yet unknown different peroxisomal protein component. This, in turn, could explain ongoing problems in expressing Pex7p as a separate protein (Schliebs et al., unpublished). Intriguingly, such insert has recently been observed in the two-component complex Sec13/31, which is involved in vesicle-coated export of proteins from the endoplasmatic reticulum [18]. In this complex, one WD40 motif of the Sec31 β-propeller is donated by Sec13, which mostly comprises an α-solenoid fold.

The presence of the WD40 sequence signature throughout most of the remaining Pex7p sequence and the availability of about 25 Pex7p sequences from different species (not shown) supports the localization of conserved residues as a potential indication for functional/structural relations (Fig. 3). Threading of the Pex7p sequence onto the coordinates of a protein with an experimentally determined seven-bladed propeller fold, the histone methyl-lysine recognition domain WDR5, provides insight into potential Pex7p receptor binding sites (Fig. 3A). Because of the alternating inner/outer surface pattern of loops in WD40 propeller modules [51] as well as significant sequence similarity of the structural template and the target protein Pex7p (22% identical residues), the available data allow an analysis of spatial the clustering of conserved residues. The structure-based sequence alignment indeed predicts that virtually all invariant residues are located at the inner surface loops while nearly none are found at the outer surface loops (Fig. 3A, for technical details, see legend of Fig. 3). The imbalance in the distribution of conserved residues is also illustrated on a putative structural model, which considers a complete seven-bladed WD40 propeller formed by Pex7p (Fig. 3B). Because of the uncertainty of the structural organization of the first WD40 module, we have not included the N-terminal part of the Pex7p sequence in the analysis of conserved surfaces. Taking the data together, the highly conserved inner surface of the Pex7p model suggests that it has an important functional role, as an interaction site for either PTS2 cargos, Pex7p co-receptors, or both. Our prediction is consistent with previous experimental findings from available WD40 β-propeller structures, in which most of the protein–protein interactions involve the central part of the inner face of the disk-shape structures [51].

Ultimately, the precise identity of the interactions partners remains to be determined by experimental methods. A key aspect for future structural/functional research on the Pex7p receptor concerns assessment of whether the receptor is capable of folding on its own into a defined 3D structure of a WD40 propeller or whether it requires the presence of additional protein components, reminiscent of early findings on heterotrimeric G proteins, in which the WD40 β-propeller containing β-subunit minimally requires the presence of the additional γ-subunit. If the latter scenario proves to be correct, Pex7p co-receptors comprise the prime candidate partners.

2. Conclusions

Translocation of peroxisomal matrix proteins is carried out by two import receptors, Pex5p and Pex7p, the latter of which functions in close conjunction with co-receptors. Although, to date, structural data are limited to the CB domain of the Pex5p receptor, comparative analysis using fold predictions of the Pex7p receptor reveals an overall scenario of two receptors with complimentary structures and functions, both sharing ring-like structural arrangements. While experimental data indicate that conformational dynamics are an intrinsic property of the CB domain of Pex5p, the Pex7p receptor, in contrast, is predicted to consist of rigid β-sheet WD40 repeats. Of interest is that even such an arrangement may undergo limited conformational changes, as indicated by the structure of the heterotrimeric G protein/phosphoducin complex [36].

Furthermore, present experimental data on cargo binding to Pex5p and prediction of cargo recognition by Pex7p indicate that both receptors may bind the respective cargos by central holes or tunnels, provided by their disk-shaped CB domains. Recognition of peroxisomal proteins by central binding ''tunnels'' raises the possibility of common general principles governing the recognition of peroxisomal proteins, which have yet to be elucidated experimentally. Future experimental approaches are also needed to clarify the reason as to *why* nature has evolved two distinct import receptors, Pex5p and Pex7p, which share the same protein docking components for membrane association. One plausible explanation is the existence of specific protein cargo requirements, which may support recognition by only one of the import receptors. This view may be reinforced by specific structural requirements in some cargos, such as their state of oligomerisation and co-factor binding, that support recognition by peroxisomal import receptors [58].

Remarkably, there is an increasing number of exceptions that cannot be explained by a simple model that considers two cognate receptors for two distinct import signaling sequence motifs, PTS1 and PTS2. Why are there peroxisomal proteins, such as Pex8p from *H. polymorpha* [60], harboring both PTS motifs, which may not both be functional? How can we explain taxonomic differences in some Pex5p-dependent peroxisomal proteins, such as alcohol oxidase [23], which may or may not carry a C-terminal PTS1 motif? What is the molecular basis of other Pex5p-dependent proteins, such as acyl CoA oxidase or carnitin acyltransferase [29], which may be translocated even in the absence of the complete C-terminal CB domain? In order to address these questions, it remains an important task to unravel the structures of the receptors Pex5p and Pex7p in the presence of cargos with different requirements for cargo translocation. Although, we have begun to gain insight into the molecular principles underlying cargo recognition by the Pex5p import receptor, most of the following events in protein translocation through the peroxisomal membrane, such receptor docking, cargo release, and receptor recycling, still remain to be elucidated.

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