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Review

Dynamic architecture of the peroxisomal import receptor Pex5p

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Abstract

The majority of peroxisomal matrix proteins are recognized by the import receptor Pex5p. The receptor is dynamic in terms of its overall architecture and association with the peroxisomal membrane. It participates in different protein complexes during the translocation of cargos from the cytosol to the peroxisomal matrix. Its sequence comprises two structurally and functionally autonomous parts. The N-terminal segment interacts with several peroxins that assemble into distinct protein complexes during cargo translocation. Despite evidence for α-helical binding motifs for some of these components (Pex13p, Pex14p) its overall appearance is that of a molten globule and folding/unfolding transitions may play a critical role in its function. In contrast, most of the C-terminal part of the receptor folds into a ring-like α-helical structure and binds folded and functionally intact peroxisomal targets that bear a C-terminal peroxisomal targeting signal type-1. Some of these targets also bind to secondary binding sites of the receptor.

Keywords: Receptor; Structure; Cargo import; Peroxisomal targeting signal; Translocon

1. Introduction

Import of peroxisomal matrix proteins fundamentally differs from most other protein translocation systems identified and characterized to date [35,53]. Firstly, it allows the translocation of folded, functional and assembled protein cargos. Secondly, since there is no evidence for a constituent peroxisomal translocon complex at present, it is generally assumed that such translocon is of the signal-assembled class [53] and of transient nature [18,22,49,57]. Because of the difficulties of capturing such a complex, details of its composition, function and structure have so far remained scarce.

Most of the soluble protein targets destined for translocation into the peroxisomal matrix are recognized by the receptor Pex5p [10]. It generally recognizes targets with a C-terminal type 1 peroxisomal targeting signal (PTS1) motif [18,22,49,57]. However, ever more Pex5p dependent targets where the presence of this motif is not essential or even absent are being discovered [15,23,26,31,43,44,51]. In higher vertebrates, the receptor exists in multiple isoforms. The long Pex5pL version differs from Pex5pS by an insert of 37 residues, encoded by a single extra exon, with a Pex7p receptor binding site that connects the longer isoform to PTS2 import [4]. Another isoform, termed Pex5pM, which is seven residues shorter than Pex5pL, has recently been identified in CHO cells [25]. Common to many lower eukaryotes, in which only one Pex5p isoform has been detected, is the presence of auxiliary peroxins, such as Pex18p and Pex21p in *S. cerevisiae* or Pex20p in *Y. lipolytica* and *H. polymorpha* [13,41,51]. These peroxins apparently substitute the participation of the Pex5pL isoform in PTS2 cargo import.

There is accumulating evidence that the Pex5p receptor participates in virtually all major steps of PTS1 target import into peroxisomes, including target recognition, target translocation, target release and recycling of the receptor—thus, PTS1 target import could be regarded as synonymous with the Pex5p cycle. Since the receptor does not operate as a separate translocon it is not surprising that it is involved in several multi component protein complexes during the target translocation process. The well-studied docking complex is responsible for attachment of the target bound receptor on the *cis* side of the peroxisomal membrane and comprises, in addition to the receptor, minimally the peroxins Pex13p and Pex14p [22,57]. In

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lower eukaryotes, in addition, Pex8p and Pex17p have been identified to play a role in docking and translocation [1,19,51]. A second group of complex components (Pex2p, Pex10p, Pex12p) have a RING finger domain in common and seem to be involved in the translocation process across the peroxisomal membrane. In *S. cerevisiae* and *P. pastoris*, the two assemblies appear to be linked by Pex8p that functions as a PTS1 cargo on its own and leads to dissociation of other cargos from the receptor [1,63,67]. Finally, Pex4p mediated Pex5p receptor ubiquitination and ATP consuming processes involving the membrane-associated AAA ATPases Pex1p and Pex6p seem to play a critical role during target dissociation at the trans-side of the peroxisomal membrane and controlled receptor recycling [28,33,47,48]. Taken together, an overall picture of the receptor emerges that, from a structural perspective, makes it a mandatory task to investigate its structural organization in the presence of interacting components that may lead to substantial alterations of the conformation of parts of the receptor. This may also explain why, at present, relatively little is known about its overall architecture and its interacting protein partners. The remaining part of the review will summarize presently available structural data. Unless mentioned otherwise, it will focus on the mammalian Pex5p receptor that has been used for most of the structural investigations so far. Since it shows considerable taxonomic variability in terms of sequence, structure and function, conclusions drawn from the mammalian receptor may not apply *a priori* for equivalent receptor systems from other organisms.

2. Sequence and domain organization of the Pex5p receptor

The sequence of the long isoform of the receptor Pex5pL comprises 639 residues. Both functional and structural data indicate it comprises two separate parts (Fig. 1). Sequence alignment reveals poor conservation in the N-terminal domain, while the C-terminal TPR domain is highly conserved (data not shown). The N-terminal part of Pex5p hosts most the interaction sites for other peroxins. Although an early characterization of the structural/functional properties of the receptor suggested that it may play a role in oligomerisation [52] a more recent investigation of the hydrodynamic properties of the N-terminal part of Pex5p revealed that it is in fact monomeric, with an abnormal shape that is generally not found in globular protein structures [8]. These findings may require reinterpretation of previous data and hypotheses of a 'pre-implex model' that considered the recruitment and import of target assemblies by an oligomeric receptor [18,63]. The pre-implex model does not *per se* require homo-oligomerisation of Pex5p, only sufficient kinetic stability of a Pex5p containing 'ordered aggregate', in which any number of scaffold proteins may play a role. A remarkable feature of the Pex5p N-terminus originates from a seven-fold repeated pentapeptide WxxxF/Y motif [32,39,52,66]. Some of these motifs are involved in binding to two Pex5p docking complex components, Pex13p and Pex14p [40,50,52]. Although these findings have stimulated appealing hypotheses about possible Pex5p/Pex14p stoichiometries [8,50,52] a rigorous quantitative determination of the competitive and/or complementary involvement of these motifs to both peroxins, Pex13p and Pex14p, still remains to be carried out. The only moderate conservation of these motifs in available Pex5p sequences directs to taxonomic variations of these interactions. Taxonomic specification is supported by recent data indicating, that in *S. cerevisiae* Pex14p binding to the Pex5p receptor may not be dependent on the presence of WxxxF/Y motifs [65]. Additionally, it has been observed that deletion of *S. cerevisiae* PEX5 can be complemented by chimeric Pex5p comprised of the N-terminal domain of *S. cerevisiae* Pex5p and the relatively highly conserved C-terminal domains from human, tobacco and nematode [20]. One report shows that heterologously expressed watermelon PEX5 restores peroxisome formation in the methylotrophic yeast *H. polymorpha*, but that PTS1 import is only partially restored [66], further supporting the suggestion of taxonomic specification.

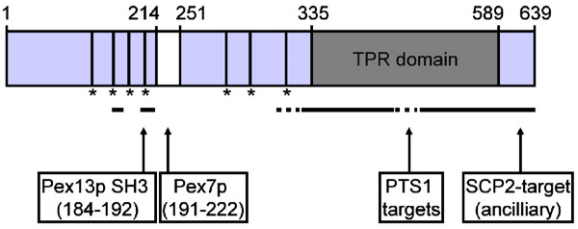

Fig. 1. Schematic presentation of the domain structure of the human Pex5pL receptor and interaction sites for known components of the Pex5p docking complex. Residue numbers correspond to the mammalian Pex5pL sequence. Locations and significance of some interactions sites are under active discussion (see text). The seven horizontal bars marked with (*) indicate WxxxF/Y Pex14p binding motifs (residues 118–122, 140–144, 159–163, 184–188, 243–247, 257–261 and 308–312). The white area indicates the extra 37 residues absent from Pex5pS. Those sequence parts of the Pex5p receptor where high resolution structural data are available ([12,17,45,46,56]; Neufeld, unpublished) are indicated by the bar at the bottom of the figure, with the dotted segments of the bar indicating residues that were present during crystallization but are not visible in the crystal structures. For further details, see text.

The key function of the C-terminal part of the Pex5p receptor is to recognize PTS1 motif containing protein targets, as originally evidenced by deletion and mutagenesis studies [5,9,30,58]. Several mutations of the Pex5p receptor with pathological effects for peroxisomal import have been linked, at the molecular level, to defects in recognizing PTS1 motif containing targets [4,54,64]. Differential binding affinities of the C-terminal PTS1 motif have been quantitatively investigated both by computational and by experimental approaches, demonstrating the importance of the three C-terminal residues as well as some of the preceding residue positions for Pex5p receptor binding [36–38]. In addition, there is accumulating evidence that a subset of PTS1 contains a second, ancillary recognition site [23,56]. For some PTS1 targets, recognition by the Pex5p receptor seems to be regulated by activating protein partners or chaperones [21,43]. Taking these data together, a model of PTS1 cargo recognition by the Pex5p receptor emerges in which residue-specific interactions from the C-terminal PTS1 motif are critical for receptor binding but not sufficient in the context of folded, functional cargos. While a number of processes have been investigated that lead to receptor recognized PTS1 targets, such as oligomerisation and

cofactor binding [21,43,59], it remains largely elusive, *vice versa*, what kind of molecular parameters within the cargo structures *inhibit* their recognition by the Pex5p receptor. Finally, it is noteworthy mentioning that the Pex5p receptor recognizes some targets without the canonical PTS1 motif, however, by mechanisms that are not restricted to the C-terminal domain of the receptor [31]. The latter finding has allowed the construction of a functional Pex5p import receptor, comprising the N-terminal part only [51].

3. Structural data regarding the N-terminal part of the Pex5p receptor

Recent biophysical and biochemical data indicate that the N-terminal part of the Pex5p receptor does not behave like a globular, folded protein [6,8], rendering high resolution structural analysis difficult or even impossible. The recent conclusion that "the N-terminal half of Pex5p is best described as a natively unfolded pre-molten globule-like domain" [6] is supported by computational analysis and the same description may be applied to Pex5p proteins of different taxa. Fig. 2 plots the mean disorder propensity of 20 different Pex5p sequences, as evaluated by the program IUPred [11]. It is clear to see that the N-terminal part of Pex5p has a higher disorder propensity than the C-terminal globular domain. The relatively low sequence conservation in the N-terminal domain, compared to the C-terminal TPR domain, may be postulated to result from the absence of evolutionary pressure of natively disordered proteins to retain a globular fold [61].

It is possible to speculate on the functional significance of the apparent disorder in the N-terminal domain of Pex5p. Tompa functionally classifies natively disordered proteins [60,61] into two groups—"entropic chains" and "recognition sites". Each group can be subdivided into more specific categories but for the purpose of speculating on Pex5p, only a couple of possible functions need to be mentioned here. As an example of an "entropic chain" function, it could be hypothesized that the potential disorder–order–disorder transitions which segments of Pex5p may undergo upon sequential interactions with ligands (e.g. docking complex proteins Pex13p and Pex14p) may be of thermodynamic and kinetic importance in regulating events. The sign and magnitude of the entropy component, coupled to the rate of a molecular interaction, will have significant bearing on efficiency and transience of that interaction.

In consideration of "recognition sites", it is likely that the N-terminal domain of Pex5p exhibits a number of so-called display sites, allowing for interaction with the large number of ligands that have been found to associate with this part of the protein (Fig. 1). Ligands may be non-covalent, such as proteins (e.g. the docking complex) and peroxisomal membrane lipids [14,27], or covalent modification by ubiquitination [29]. Although statistically phosphorylation predominantly occurs in disordered regions of proteins [24] and computational prediction tools indicate that Pex5p bears multiple phosphorylation sites (data not shown), to our knowledge, there is no published empirical evidence for Pex5p phosphorylation to date.

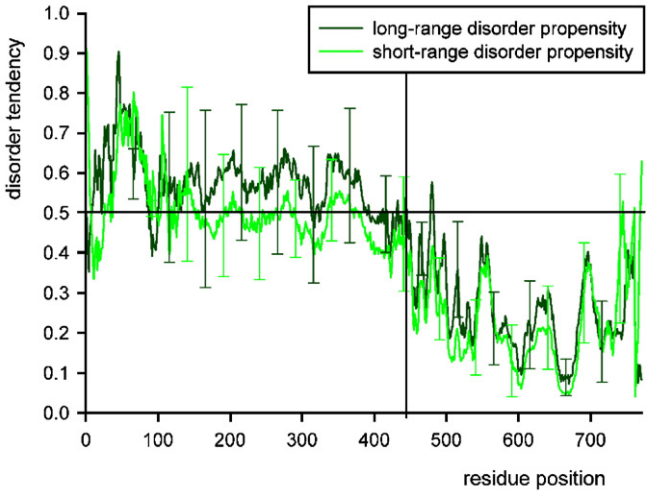

Fig. 2. Prediction that it is a general feature of the N-terminal domain of Pex5p to be natively disordered. 20 Pex5p sequences were analysed by IUPred [11] for disorder tendency over a long-range window of 100 residues (shown in dark green) and a short-range window of 25 residues (light green). Disorder propensities were compared using the first residue of TPR1 as a fixed point (corresponding to His335 in human Pex5pL, the position shown by the black vertical line) and the mean disorder tendency calculated. Thus, the *x*-axis, residue position, covers the length of the longest Pex5p sequence included, that of *Arabidopsis thaliana* (728 residues). Standard deviations are shown with bars the same color as the corresponding mean curve. A threshold disorder propensity of 0.5 is regarded as a high tendency towards non-globularity. The 20 sequences used were from *Homo sapiens*, *Cavia porcellus*, *Cricetulus griseus*, *Mus musculus*, *Rattus novegicus*, *Brachydanio rerio*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Citrullus lanatus*, *Nicotiana tabacum*, *Arabidopsis thaliana*, *Leishmania donovani*, *Trypanosoma brucei*, *Penicillium chrysogenum*, *Neurospora crassa*, *Yarrowia lipolytica*, *Hansenula polymorpha*, *Pichia pastoris*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

Based on findings from the Distel laboratory for a distinct Pex13p binding site in the Pex5p receptor from *S. cerevisiae*, which is not competitive for binding to Pex14p [2,3], structural studies of the corresponding peptide segment from Pex5p in the presence of the Pex13p SH3 domain were carried out [12,45,46]. The Pex5p segment interacts with a site in the Pex13p SH3 domain that is opposite to the canonical PxxP binding site and, although disordered when free in solution, adopts an α-helical conformation upon binding. The binding affinity was measured to be 36 μM, using a synthetic receptor peptide, spanning residues 198–214 of Pex5p from *S. cerevisiae*. A complementary crystal structure showed that a PxxP motif containing sequence motif from the docking factor Pex14p from *S. cerevisiae* indeed binds into the canonical ligand binding site of the Pex13p SH3 domain [12]. Additional functional data, however, revealed, in terms of taxonomic divergence, binding effects induced by the presence of cargos and led to the identification of different binding sites, both in the Pex5p receptor and in the Pex13p docking factor [40,62]. Therefore, the presently available structural models may not be generally applicable as a basis of the Pex5p–Pex13p interactions in peroxisomal docking complexes. A recent NMR structure of one of the WxxxF/Y motifs of the Pex5p receptor with another docking complex component, the N-terminus of Pex14p, has revealed an α-helical conformation

of the motif in the presence of Pex14p as well (Neufeld et al., unpublished).

4. Structural data regarding the C-terminal part of the Pex5p receptor

In contrast to the N-terminal part of the receptor, the C-terminal part can be purified as a stable, soluble domain. Its structure has been characterized by solution scattering [55] and crystallized, in the presence or absence of cargos [17,56]. While the repeated presence of tetratricopeptide repeat (TPR) motifs and the involvement of these motifs in PTS1-cargo recognition was realized prior to any available high resolution structural information [5,9,30,58,52]) several crystal structures of the C-terminal part of the receptor revealed its molecular architecture [17,56]—as shown in Fig. 3. The structures show how two TPR triplets generated from TPR sequence motifs 1–3 and 5–7 arrange into a ring-like structure. In contrast, the sequence accounting for the fourth TPR motif is only partly visible and displays a distorted conformation. Initial doubts about the identity of the motif and the connectivity of the two TPR triplets [17] could be clarified by recent structural data that support the role of the TPR4 domain as a conformationally mobile unit [56]. The potential of conformational flexibility within the TPR motifs of the Pex5p receptor has also been demonstrated by a crystal structure of the N-terminal TPR triplet, in which the third TPR motif opens in a jack-knife type of motion, supported by the presence of a single magnesium ion [34]. In addition to the seven-fold repeated structure of TPR motifs, the structure of the Pex5p receptor/peptide complex contains a C-terminal bundle formed by three antiparallel helices, which did not appear to be involved into binding of the PTS1 receptor recognition motif.

Comparison of the structures of the receptor in the presence and absence of a complete and functional protein cargo, sterol carrier protein 2 (SCP2), has allowed to unravel some of the molecular parameters that are critical for cargo recognition by Pex5p. Upon binding of SCP2 the overall conformation of the C-terminal part of the receptor changes from an open snail-like conformation, established by the sequence of the seven TPR domains, into a closed ring-like conformation. The closure of the latter conformation is established by a long loop, referred to as to '7C-loop' [56], that inserts between the domains modules TPR1 and TPR7 while the same loop largely disassembles from the remaining structure in the absence of SCP2. *In vivo* loss-of-function and localization assays have confirmed the critical contribution of several residues from this loop in cargo (SCP2) recognition. Comparison of the available structures of the C-terminal part of the receptor in the presence of a PTS1 peptide and functional cargo SCP2 [17,56] shows a virtually identical conformation of the receptor, suggesting that the observed changes, taking the apo-structure of the receptor as reference, are induced by the C-terminal PTS1 motif rather than by other parts of the cargo. Based on the previous structure of the receptor-PTS1 peptide complex [17] the contributions of

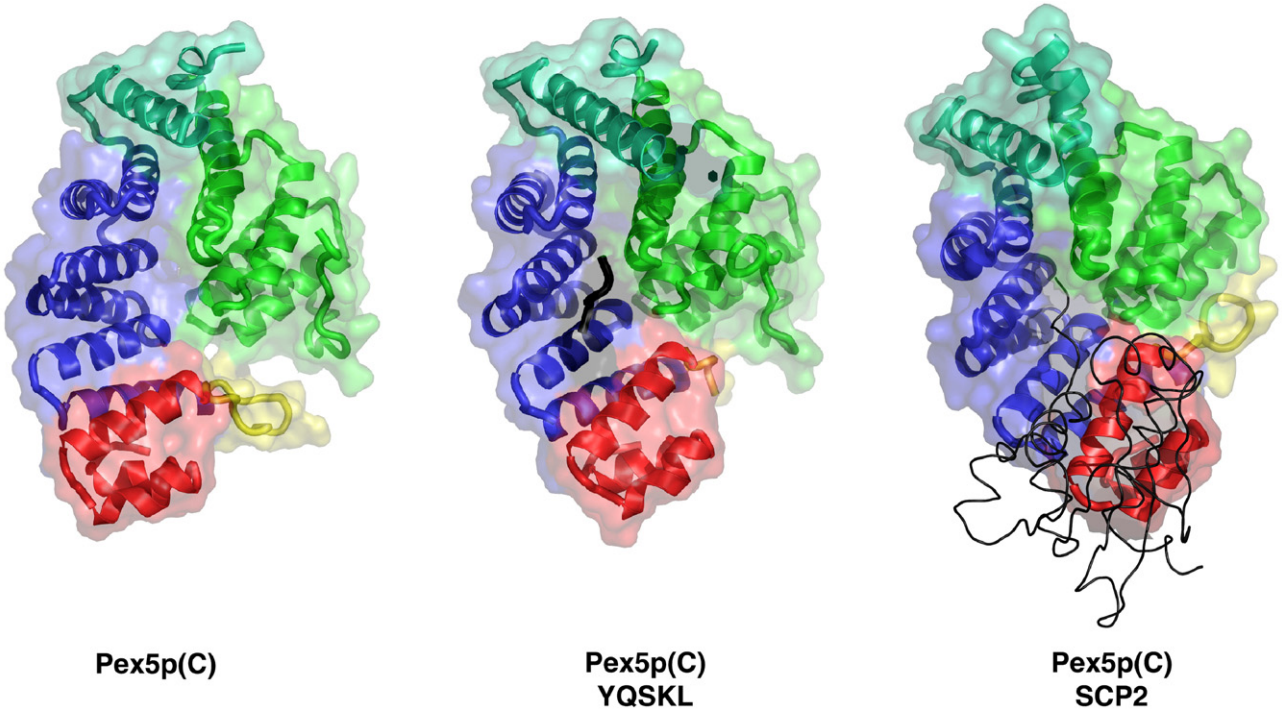

Fig. 3. Available Pex5p(C) structures, in the absence of any ligands (left, [56]), in the presence of the consensus PTS1 peptide YQSKL (central, [17], 1FCH), and in the presence of the functional PTS1 cargo protein SCP2 (right, [56]). Color coding: TPR segments 1–3, green; TPR4 segment, turquoise; TPR segments 5–7, blue; loop connecting the TPR segment and the C-terminal helical bundle, referred as to '7C-loop' in [56], yellow; C-terminal α-helical bundle, red; PTS1 ligands, black. While the two liganded structures bear virtually identical ring-like arrangements amongst their helical domain elements, unliganded Pex5p(C) is in a distorted snail-like conformation, due to disruption of contacts between the 7C-loop and the TPR1 segment. In addition to the PTS1 binding site at the central groove that is formed by the ring-like arrangement of the seven TPR segments of the Pex5p receptor, SCP2 forms a secondary interface with the C-terminal helical bundle of Pex5p receptor.

several terminal PTS1 motif residues were tested and validated, either empirically or by predictions [37,38].

Furthermore, the recent structure of the Pex5p–SCP2 complex locates a second cargo binding site, situated on the surface of the C-terminal helical bundle of the receptor. *In vitro* binding data estimate an five-fold increase of the binding affinity by the presence of the folded, functional domain of the cargo when compared to the affinity of the C-terminal PTS1 motif only [56]. Mutations within this secondary interface, however, lead to effects that cannot be simply interpreted as loss-of-function effects (Stanley et al., unpublished). Whether this site may play a role in cargo sorting or even cargo release, for instance, is under investigation, at present.

5. Future perspectives

Given the central involvement of the Pex5p receptor in import of protein targets into peroxisomes, surprisingly little is known about its structural organization. Although the molecular basis of the recognition of at least one cargo destined for translocation into peroxisomes has been characterized [56] it remains unknown, at present, whether the ancillary binding site of the cargo SCP2 is generally employed for binding of other PTS1 cargos as well or whether there are different auxiliary cargo binding sites on the Pex5p receptor. Although SCP2 and a few other cargos can be bound to the Pex5p receptor *in vitro* in our laboratory's and others experience the binding of several complete cargos seems to be inhibited, even when the corresponding isolated C-terminal PTS1 peptides do bind [15] (Schüller et al., unpublished). These data indirectly support previous evidence of the involvement of additional 'activating' factors for recognition of some cargos by the Pex5p receptor. Moreover, although direct comparison of the structures of the prototype cargo SCP2, bound and unbound to the receptor, indicate a disassembly process of the C-terminal PTS1 motif from the core fold of the cargo [7,16], little is known to date about the molecular mechanisms of this process. We also hypothesize, given that many characterized PTS1 cargos assemble as oligomers, that changes in their oligomerisation states may be involved in receptor recognition by blocking/unblocking C-terminal PTS1 as well as potential ancillary receptor recognition motifs. In summary, in the light of our and previous data on structures of complexes of the C-terminal part of the Pex5p receptor and at least one complete cargo (SCP2), there is a promising perspective to determine the molecular structures of further C-terminal receptor/cargo complexes by making use of established protocols to allow to unravel general principles of PTS1 cargo recognition by the receptor.

However, some of the key questions to understand functional/structural relationships of the receptor still remain unresolved, specifically: what kind of structural dynamics of the receptor are involved in the process of translocating the receptor from an initial docking complex and into complex that allow release of the cargo? What kind of conformational transitions, probably accompanied by receptor ubiquitination and/or other modifications, are involved in the recycling process of the receptor? The key challenge for future structural characterization seems to be on the participation of the Pex5p receptor into a multi-component translocon or importomer [1], which is thought to be induced by the presence of cargo signals for translocation and, therefore, difficult to capture as a constitutive assembly. Hence, a key research aim will be to develop protocols to capture previously suggested stages of such Pex5p containing importomer for direct visualization and potential molecular structural analysis.

The hypothesis of the involvement of Pex5p in an inducible translocon or importomer is supported by increasing evidence that the N-terminal part of the receptor to behave as non-globular protein with 'molten globule' features in the absence of further interacting protein components [6,8]. Although several protein components of complexes involving the Pex5p receptor have been identified and characterized, most likely the data are still far from being complete and questions remain about their sequential and/or parallel organization. Therefore, in our estimate, the most likely way to advance structural knowledge of the receptor will be to attempt to determine complex structures of the receptor in the presence of identified scaffold components such as, for example, Pex13p and Pex14p. Such structural data may be acquired in the presence or absence of cargos, which potentially may be useful as vehicles to grow diffracting crystals. Another promising route may be on the effects of cross-stabilization of different Pex5p isoforms or components (Pex18p/Pex21p and Pex20p) that may substitute Pex5p function in lower eukaryotes [13,42,51]. This approach may require the development of suitable co-expression protocols and separate structural approaches on interacting components. Although some initial data are available on separated domains from these scaffolds [12,46] (Neufeld et al., unpublished) there is increasing evidence that the assumptions, which have been made for future structural investigations of the Pex5p receptor, equally apply for many other peroxins as well: they function in complexes and they need to be treated as such, in order to advance the knowledge of their underlying molecular architectures.

Acknowledgments

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