Review of: "The Structure of the Arabidopsis PEX4-PEX22 Peroxin Complex—Insights Into Ubiquitination at the Peroxisomal Membrane"

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Structure and activity of the UBC peroxin complex in *Arabidopsis thaliana*.

A recent study by Traver *et al.* [1] describes the X-ray crystal structure of the *Arabidopsis thaliana* ubiquitin-conjugating enzyme PEX4 complexed with the C-terminal part of its membrane-adaptor protein PEX22. Moreover, the authors present a bichemical analyzis
of the PEX4-PEX22 interaction and ubiquitin-conjugating activity.
This commentary highlights the main results of the study and discusses their possible implications concerning the work on the function of peroxisomes.

Peroxisomes are organelles that play a central role lipid metabolism and related oxidation-based pathways. The functionality of peroxisomes depends on the proper compartmentalization of their enzymes into the organellar matrix. The import of peroxisomal matrix proteins occurs posttranslationaly and depends on the presence of
their peroxisomal targeting sequence (PTS). The soluble PTS receptors recognize the PTS-containing proteins in the cytosol and ferry them to the peroxisomal membrane, where the PTS proteins are thought to be released into the peroxisomal matrix via a transient import pore. Finally, the PTS receptors are returned to the cytosol by the AAA+ peroxins, a hetero-meric ATPase complex. The recognition signal is the ubiquitination of the PTS receptors. Polyubiquitination via K48-linked chains on lysine residues primes the receptors for proteasomal degradation, while monoubiquitination on a conserved cystein leads to
the recycling of the receptors for further rounds of protein import. The monoubiquitination is catalyzed by the peroxisomal UBC complex, consisting of the ubiquitin-conjugating enzyme (E2) PEX4 and its membrane-adaptor PEX22, in cooperation with the peroxisomal ubiquitin-protein ligase (E3) complex, composed of PEX2, PEX10 and PEX12 [2-3]. Therefore, the PEX4-PEX22 module has a central function in the mechanism of peroxisomal matix protein import.

The first X-ray crystal structures of plant peroxins

The study by Traver *et al.* [1] presents the first structural data of plant peroxins. Moreover,
it provides important pieces of information that add up to the results from the already published structures of PEX4-PEX22 from the yeasts *Saccharomyces cerevisiae* [4] and *Ogataea angusta* (formerly known as *Hansenula polymorpha*) [5]. The

comparison shows that *A. thaliana* PEX22 lacks significant amino acid sequence similarity to the yeast orthologs [1]. However, the structural data reveal that all analyzed PEX22 proteins share a Rossman fold-like structure [1]. Therefore, it is the structure of the binding area that seems to be conserved. It will be interesting to see if this is also the explanation for the surprisingly low sequence similarities between the orthologs of the membrane anchor protein of the AAA+ peroxin complex, which is called Pex15 in yeast, PEX26 in mammals or APEM9 in plants [3,6]. Future structural studies might reveal that not the amino acid sequence but the functional fold is conserved between the AAA+ peroxin anchor proteins.

Moreover, the *A. thaliana* structures show that the association of PEX4 and PEX22 is supported by electrostatic interactions and therefore has to be very specific. Thus, it seems unikely that other UBCs, like the ones involved in PTS receptor polyubiquitination, might also bind to PEX22. In addition, it was shown for PEX4 that the PEX22-binding site does not overlap with the corresponding binding sites for the E1 and E3 enzymes [1].

This indicates an efficient and specific mode of ubiquitination that would allow a fast modification of the import pore-bound PTS receptors for efficient recycling or other potential targets for rapid degradation.

Ubiquitination activity of the PEX4-PEX22 module

The physiologic modification required for the recycling of the PTS receptors, like yeast PEX5 or PEX18/PEX20, is the monoubiquitination of the conserved cysteine-residue, which is catalyzed by PEX4 [3]. In contrast, the PTS receptors are marked for proteasomal degradation with K48-linked polyubiquitin chains by more promiscuous enzyme like UBC4 [3]. In the case of PEX4-catalyzed monoubiquitination of PTS receptors, it has been demonstrated that PEX4 displays full activity in the presence of PEX22, which therefore functions not only as membrane anchor but also as an allosteric activator [4-5,7]. As recently shown in the *A. thaliana* study [1] and *O. angusta* work [5], PEX4 can undergo autoubiquitination during *in vitro* assays. The finding that it builds K48-linked polyubiquitin chains in the presence of the C-terminal part of PEX22 might suggest that this result may not only be found *in vitro* but could potentially also apply to *in vivo* targets. One possibility would be that under certain circumstances PEX4 could also polyubiquitinate the PTS receptors. This was suggested by the observation that the destabilized PEX5 of the *A.thaliana pex6-1* mutant was stabilized when combined with the *pex4-1* mutant [8]. However, this effect could also be indirect, as PEX4 has been described to be directly involved the autoubiquitination of the three yeast RING peroxins [9] and the destabilization of the *A.thaliana pex12-1* mutant of PEX12 [10]. Therefore, PEX4 could possiby be involved indirectly on the level of PEX5 polyubiquitination and directly on the level of RING peroxin polyubiquitination, which would represent a relevant and not well understood regulatory mechanism.

Elongated unstructured tether of PEX22: a molecular fishing line to reach distant targets?

Another interesting aspect is the finding that *A. thaliana* PEX22 displays an unstructured tether between the N-terminal transmembrane domain and the C-terminal PEX4-binding area. This felxible region could allow PEX4 to remain bound to PEX22, while being able to associate with different targets in its surrounding. This point might also impact the work on other open questions regarding functional positioning of PEX4-PEX22 at the peroxiomal membrane. For instance, it is unknown how or if the PEX4-PEX22 module associates with the peroxisomal import machinery. Based on current data,

this is especially of interest for the yeast peroxisomes, where the PTS1- and PTS2-import pores dynamically form and dissociate independent from each other. As yeast PEX4 ubiquitinates both the PTS1-receptor PEX5 and the PTS2-co-receptors PEX18/PEX20 [3], it could mean that instead of two Pex4p-Pex22p modules only one unit would be needed, which then could reach both transient import pores via the ubiquitination of the corresponding receptors.

The amino acid sequence alignment shows that this tether is especially elongated in PEX22 of different plant species [1], suggesting an additional plant-relevant function of this region. Therefore, two roles attributed to the peroxisomal ubiquitination machinery of plants might come into focus: Developmental changes of oilseed seedlings include the regulated remodeling of the peroxisomal matrix protein composition in which ceratin glyoxylate cycle enzymes are replaced by photorespiration enzymes. The obsolete enzymes are degraded depending on the peroxisomal ubiquitination machinery [11]. It is unkown how or whether PEX4 directly binds and ubiquitinates these matrix enzymes. However, the elongated

PEX22 tether could offer more possibilities to associate with this class of targets beyond the interaction to the import receptors or the import machinery. The second point concerns the organellar contact site of peroxisomes and chloroplasts. The chloroplast-peroxisome contacts are regulated by light are thought to represent a requirement for efficient

shuttling of metabolites through the photorespiratory pathway. It has been described that the chloroplast-peroxisome association is depending on the RING-domain of PEX10 [12]. Because the nature of the contribution of PEX10 to the regulation of this contact site is unknown, it is interesting to speculate whether PEX4-PEX10-catalyzed ubiquitination processes might be involved. With the possible help of the elongated PEX22 tether more distantly positioned proteins could be targeted for ubiquitination by PEX4 - both on the peroxisome or even on the chloroplast side.

In summary, the Traver *et al.* study [1] provides important insights into the structure and activity of the peroxisomal PEX4-PEX22 module. On a more general scale, it will be of interest to analyze if these findings and concepts could also be transferred to other UBC-adaptor proteins. Especially the interplay of UBC7 and CUE1 during endoplasmic reticulum associated degradation (ERAD) is of interest, as the ubiquitination and extraction machineries of peroxisomes and ER share mechanistic similarities [13]. A more specific focus on the PEX4-PEX22 module itself would be to elucidate how the decision for monoubiquitination of the PTS receptors or the polyubiquitination of other targets is realized and executed on the structural level.

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