Review

Peroxisomal matrix protein receptor ubiquitination and recycling

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Received 10 May 2006; received in revised form 15 August 2006; accepted 23 August 2006

Abstract

The peroxisomal targeting signal type 1 (PTS1) receptor Pex5 is required for the peroxisomal targeting of most matrix proteins. Pex5 recognises target proteins in the cytosol and directs them to the peroxisomal membrane where cargo is released into the matrix, and the receptor shuttles back to the cytosol. Recently, it has become evident that the membrane-bound Pex5 can be modified by mono- and polyubiquitination. This review summarises recent results on Pex5 ubiquitination and on the role of the AAA peroxins Pex1 and Pex6 as dislocases required for the release of Pex5 from the membrane to the cytosol where the receptor is either degraded by proteasomes or made available for another round of protein import into peroxisomes.

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Keywords: AAA protein; Peroxisome biogenesis; Pex1p; Pex6p; Pex5p; Pex15p; Peroxin; Ubiquitin

Ubiquitination plays a vital role in the physiology of cells and organisms. Among the first functions of ubiquitination to be discovered was its role in the targeting of proteins for degradation by the proteasome. In the recent years, it has become more and more evident that ubiquitin attachment to proteins also serves other purposes like control of protein activities or organelar targeting. In this review, we survey the components of the ubiquitination machinery and their relevance to peroxisome biogenetic functions. The ubiquitin-proteasome system (UPS) comprises the core ubiquitination machinery E1, E2, and E3, as well as the proteasome together with AAA proteins and ubiquitin-binding and deubiquitinating proteins. Several lines of evidence point to an involvement of components of this extended UPS in peroxisome biogenesis: (1) an E2 enzyme, Pex4, is essential for peroxisomal biogenesis. (2) The AAA peroxins, Pex1 and Pex6, are likely to be responsible for the ATP-dependency of peroxisomal protein import. (3) RING proteins (Pex2, Pex10, and Pex12), which are putative E3s, are required for protein import into peroxisomes. (4) The matrix protein receptors Pex5 and Pex18 (Pex20) have been demonstrated to be modified by ubiquitination.

We suggest a model in which Pex4-dependent receptor mono- or polyubiquitination is required for functional AAA-dependent receptor release followed by the recycling or degradation of the receptors depending on the type of ubiquitination.

1. Ubiquitination basics

The UPS involves ubiquitin attachment and proteasome degradation of ubiquitinated proteins (reviewed in [1]). For ubiquitin attachment, ubiquitin is activated in an ATP-dependent manner by a ubiquitin-activating enzyme (E1). It is transferred to a ubiquitin-conjugating enzyme (Ubc, E2) that – supported by a ubiquitin-protein ligase (E3) – attaches ubiquitin to ε-amino groups in target protein lysine residues or the ε-amino group of the N-terminal residue. The conjugated ubiquitin itself may become an ubiquitination substrate in a process called polyubiquitination. The attachment of at least four ubiquitin units is required to target proteins for degradation by the proteasome. Usually, lysine at position 48 of ubiquitin is...
ubiquitinated in polyubiquitination. The term ‘monoubiquitination’ refers to the modification of a protein by one or several (‘multiubiquitination’) ubiquitin moieties which themselves are not ubiquitinated.

Target protein recognition relies on the interaction of one of a few E2 enzymes with one of many E3 enzymes. Of the eleven ubiquitin specific E2 enzymes in yeast, the similar proteins Ubc4 and Ubc5 are least specialised and required for degradation of unstable proteins [2]. Three types of E3 enzymes are known so far. (a) HECT (homologous to E6-AP C-terminus) proteins, (b) RING (really interesting new gene) gene products, or (c) U-box proteins (reviewed in [3]). HECT domain proteins bind ubiquitin to a cystein residue in their HECT domain before transferring it to the target molecule, whereas RING and U-box domain proteins facilitate the interaction between E2s and the target without previous covalent binding of the ubiquitin.

Whereas polyubiquitination of proteins classically serves as a degradation signal, monoubiquitination can be a targeting signal (reviewed in [4]). Monoubiquitin attached to plasma membrane proteins is a signal for their internalisation into the endocytic pathway. Similarly, monoubiquitin is associated with membrane protein uptake in late endosome vesicles (multivesicular bodies) for delivery into the lysosome or vacuole (reviewed in [5]). Other examples are p53 and Rad18 that are either monoubiquitinated for export from the nucleus, or they are polyubiquitinated for proteasomal degradation [6,7]. Monoubiquitination of histones is required for mitotic cell growth and meiosis and regulation of eukaryotic transcription activation [8,9].

The identification of Pex4/Ubc10 (formerly Pass2) as an – at the time putative – E2 in peroxisome biogenesis marked the first association of the ubiquitination system with peroxisome biogenesis [10]. Pex4 is membrane-anchored through Pex22 in yeast and plant [11,12]. Cells lacking Pex4 are characterised by the absence of peroxisomes with a typical morphology and mislocalisation of peroxisomal matrix proteins to the cytosol. Deletion of PEX4 or PEX22 also leads to a decrease in Pex5 abundance [11–14].

2. AAA-type ATPases

AAA proteins are ATPases that have originally been named after the identification of Pex1 (previously called Pas1) as “ATPases associated with various cellular activities” [15]. By inclusion of a more varied number of metabolic and transcriptional regulators, the classical AAA family has been expanded and termed AAA+ family of ATPases [16,17].

AAA+ proteins are involved in protein degradation, DNA replication, membrane fusion, or the movement of microtubule motors in eukaryotes, or thermotolerance in bacteria, plant and fungi (reviewed in [18,19]). The major characteristic of the AAA family of proteins is the presence of one or two 200–250 amino acid ATP-binding domain(s) that contain(s) Walker A and B motifs, as well as other motifs that distinguish the AAA proteins from other P-loop (phosphate-binding) NTPases. The defining AAA domain is a structurally conserved ATPase domain that assembles into oligomeric rings and undergoes conformational changes during cycles of nucleotide binding and hydrolysis [19].

Most of the AAA-proteins are involved in the manipulation of proteins and protein complexes leading to their unfolding or disassembly. Examples in this respect are NSF and Cdc48/p97. NSF (N-ethylmaleimide-sensitive factor) is involved in the disassembly of SNARE (α-SNAP receptor) complexes which is essential in the process of intracellular membrane fusion [20]. NSF is ubiquitously expressed in eukaryotes, but most abundant in the nervous system where it enables the membrane trafficking required for synaptic exocytosis. Cdc48/p97 is required for the dislocation of misfolded proteins from the ER substrates that are exported from the ER for subsequent proteolytic degradation through the ERAD (ER associated degradation) pathway (reviewed in [21,22]). ERAD also regulates the degradation of hydroxymethylglutaryl CoA-reductase [23] or the Δ9-fatty acid desaturase in yeast [24]. ERAD substrates are usually polyubiquitinated before they are recognised by Cdc48/p97. Golgi reassembly [25] and nuclear fusion [26] are fusion processes that are dependent on Cdc48/p97.

AAA proteins are often hexamers in their physiologically active form. In the hexameric configuration, the ATP-binding site is positioned at the interface between the subunits. The hexameric enzymes have a central pore or cavity whose function is still a matter of debate. Upon ATP binding and/or hydrolysis, AAA enzymes undergo conformational changes in the AAA-domains as well as in the adjacent N-domains that can be transmitted to substrate proteins and might lead to their unfolding or disassembly [19]. One of best-studied examples in that respect is HslU for which four conformations of the AAA+ domain could be delineated from several X-ray structures [27–29]. In HslU, the N- and C-terminal subdomains move towards each other upon nucleotide binding and hydrolysis. In the nucleotide-free state, they are most distant, whereas in the ADP-bound state, they are closest. Nucleotide binding thereby affects the opening of the central cavity of HslU, a feature that might be common to all hexameric AAA-ATPases.

AAA proteins are also crucial components of 19S cap of the 26S proteasome. The cap consists of a lid that recognises and deubiquitinates polyubiquitin-tagged substrates. The cap ATPases initiate unfolding of the substrate proteins at unstructured regions and deliver them to the central chamber of the 20S core for degradation [30,31]. AAA proteins are also involved in the disassembly of protein aggregates. Proteins that fail to fold properly often form aggregates, which might become toxic. Such aggregates can be disassembled by Hsp104/ClpB and then become substrates for refolding by the Hsp70 system [32,33].

3. The AAA peroxins Pex1 and Pex6

It was recognised from early on that peroxisomal protein import is energy dependent. In permeabilised CHO cell systems, PTS1 import was demonstrated to depend on ATP, but not on GTP or the presence of a membrane potential [34,35].
ATP-requiring was also found for import of firefly luciferase into mammalian peroxisomes [36] or of glycolate oxidase into glycosomes [37].

The peroxins Pex1 and Pex6 were identified as two ATPases of the AAA-protein family that are required for peroxisomal matrix protein import [15, 38]. Because they are the only ATPases among the known peroxins, it is likely that they are responsible for the overall ATP-requirement of peroxisomal matrix protein import [39]. Other ATP-consuming factors as well as GTPases have been found to be associated with peroxisome maintenance and movement. Dynamin-like proteins are GTPases that are proposed to play a role in peroxisome fission and inheritance [40], the Rho1 GTPase might link peroxisome to the cytoskeleton [41]. A dynein AAA+ protein as well as a kinesin have been demonstrated to work together in moving peroxisomes within the cell [42,43]. Also an Hsp70 family member and J-domain proteins are functionally associated with peroxisomes [44,45].

In their domain structure, Pex1 and Pex6 are similar to other AAA proteins with two C-terminal AAA cassettes. In both AAA peroxins, the second AAA domain (D2) is evolutionarily better conserved than the first one, which exhibits less sequence similarity to the D1 of other AAA family members. The largest differences in the two AAA peroxins can be found in their N-terminal regions. The mouse Pex1 N-domain consists of two structurally independent lobes separated by a shallow groove [46], which might provide a protein binding site.

Pex1 and Pex6 have been demonstrated to interact in an ATP-dependent manner [47–50]. However, the exact molecular constitution of this Pex1–Pex6-heterooligomer is still unclear. Cells lacking Pex1 or Pex6 are characterised by the mislocalisation of peroxisomal matrix proteins to the cytosol [15,51,52] and the presence of peroxisomal membrane ghosts which contain residual amounts of matrix proteins [53]. Interestingly, mutations in the AAA peroxins represent by far the most frequent cause of human peroxisomal biogenesis disorders (PBDs) [54].

Pex15 and Pex26 have been identified as peroxisomal membrane anchors for Pex6 in yeast [55] and mammalian cells [56], respectively. Mutational analysis of the Walker A and B motifs of Pex6 indicated that ATP-hydrolysis in the conserved AAA-domain is required to disconnect Pex6 from Pex15. Based on this data, it has been proposed that Pex6 might exert its function by an ATP-dependent cycle of recruitment and release to and from Pex15 [55]. In the Yarrowia lipolytica and Pichia pastoris, it was found that Pex1 and Pex6 are associated with peroxisomal precursors [57] or different cellular structures [49], respectively. In human fibroblasts – but not in yeast – peroxisomal localisation of PEX26 and PEX6 has been reported not to be absolutely required for AAA peroxin function [58].

The following functions have been attributed to the AAA peroxins [39]: (1) separation of the import receptor from its cargo immediately before import (‘preimperx’ model) [59], (2) dislocation of the PTS1 receptor from the peroxisomal membrane to the cytosol for recycling or degradation [60], (3) peroxisome fusion [61], (4) lipid ferries on their way to the peroxisome [62]. These suggestions are, however, not all mutually exclusive. It would be especially rewarding to conciliate the function of AAA peroxins in peroxisome fusion with their function in import receptor recycling, on which recent effort has concentrated.

4. Ubiquitin-binding proteins and deubiquitination

In the recent years, many ubiquitin-binding proteins have been identified. These include the UBA (ubiquitin-associated) domain-containing proteins as the largest family. UIM (ubiquitin-interacting motif), GAT (GGA and TOM1 proteins), CUE (coupling of ubiquitin conjugation to endoplasmic reticulum degradation), and NZF (nuclear protein localisation gene 4 zinc finger) domain-containing proteins appear to have more specialised functions (reviewed in [63]). The requirement for ubiquitin binding factors is illustrated by the AAA chaperone Cdc48/p97. To perform its function, it associates with one of several UBX (ubiquitin-related domain) proteins [64] or with the cofactors Ufd1–Npl4, Shp1/p47, Rad23, or VCP135. The basic recognition of unfolded or unstructured domains by Cdc48 does not require these cofactors [65]. Recent evidence suggests that ubiquitinated proteins are escorted to the proteasome through a pathway that involves sequential interactions with the Cdc48 cofactors Ufd1/Npl4, Ufd2, and Rad23 or Dsk2 [66].

Eventually, ubiquitin is removed from proteins by deubiquitination. There are about 17 deubiquitinating proteases (DUBs) in Saccharomyces cerevisiae [67] and about 79 functional DUBs in humans [68]. DUBs are grouped into ubiquitin carboxy-terminal hydrolases (UCHs) and ubiquitin-specific processing enzymes (UBPs). Some DUBs constitutively remove ubiquitin from substrates, whereas others have more specialised functions. At the proteasome, DUBs cleave polyubiquitin chains from proteins. The Drosophila DUB Fat facets regulates endocytosis by deubiquitinating the epsin homologue Liquid facets, a component of the clathrin-based endocytosis machinery [69]. The p97–p47 protein complex associates with the DUB VCP135 that is needed for Golgi reassembly [70,71]. So far, no specific ubiquitin binding proteins or DUBs have been found in association with peroxisome function or biogenesis.

5. Pex5 receptor ubiquitination

Peroxisomal import of matrix proteins can be conceptually divided in (1) binding of the peroxisomal targeting signals of import substrates to their receptors in the cytosol; (2) binding of these cargo–receptor complexes to a docking complex at the peroxisomal membrane; (3) dissociation of the receptor–cargo complexes and translocation of the cargo proteins across the peroxisomal membrane, (4) recycling or removal of the receptors. This conceptualisation might reflect a protein cascade of temporal and/or spatial steps in matrix protein import [39,72] and much recent work has been devoted to proving its validity. Pex5 and Pex7 are the import receptors for peroxisomal targeting signals type1 and 2, respectively. Pex7 in fungi acts in concert with a Pex20 protein (Pex18–Pex21 in S. cerevisiae,
Pex20 in *Y. lipolytica*, *Pichia pastoris* or *Neurospora crassa*) which is similar to the N-terminus of Pex5 that is required for the association with the membrane-bound components of the peroxisomal protein import machinery [73–77]. The import receptors shuttle between the peroxisomal membrane and the cytosol [80,81] or the receptors might even enter the peroxisome [78,79] lending support to an ‘extended shuttle model’. Evidence that Pex5 itself is integrated into the membrane [82] prompted the proposal of the ‘transient pore hypothesis’ [60], suggesting that the import receptor itself becomes an integral part of a peroxisomal translocon and that a cascade of protein–protein interactions leads to receptor ubiquitination and its ATP- and AAA-peroxin dependent recycling [60,83–85].

Pex5 can be ubiquitinated in vivo [86–88]. Polyubiquitination of Pex5 has been demonstrated to take place at the membrane and depends on Ubc4–Ubc5. Current evidence suggests that Pex5 polyubiquitination is not a prerequisite for Pex5 function in peroxisomal protein import. It is likely part of a quality control system that withdraws a non-functional fraction of the membrane-accumulated Pex5 from cycling by targeting it to the proteasome for degradation. It can be imagined that failure in the removal of Pex5 form the membrane would negatively affect import to some extent. As an alternative description of this process, the acronym RADAR for ‘receptor accumulation and degradation in the absence of recycling’ has been proposed [93]. In fact, deficiency in Ubc4 and Ubc5 leads to a partial import defect of peroxisomal matrix proteins [87], and overproduction of UbK48R in *Hansenula polymorpha* has even a more severe effect on the protein import into the organelle [89]. Interestingly, in a Pex5 K21R mutant, ubiquitination and degradation were abolished, suggesting that this conserved lysine 21 is ubiquitinated in Pex5 [89].

Polyubiquitination of Pex5 is enhanced in cells lacking ‘late’ import pathway components like the AAA peroxins or Pex4 or its membrane anchor Pex22. It had been noticed earlier that Pex5 levels are decreased when these factors are deleted [11,80,90,91]. This observation was used to establish an epistasis analysis of matrix protein import that placed the AAA peroxins as well as the Ube4–Pex4 at the very end of the peroxisomal protein import pathway [13].

In *S. cerevisiae* pex4, pex22, pex1 and pex6 mutants, Pex5 becomes polyubiquitinated and accumulates at the peroxisomal membrane. This phenomenon facilitated the discovery and functional characterisation of Pex5 ubiquitination [83,86,87]. Apparently the proteasomal disposal of polyubiquitininated Pex5 is less efficient in *S. cerevisiae* than in the other organisms, where AAA peroxin defects lead to a more pronounced reduction in Pex5 levels.

Pex18, which is part of the Pex7 receptor complex in *S. cerevisiae*, is ubiquitinated and becomes a substrate for a continuous proteasomal turnover [92]. It is thus a likely candidate for a similar ubiquitin-dependent regulation. In analogy, Pex18 turnover also requires the ubiquitin conjugating enzymes Ubc4 and Ubc5 [92]. Interestingly, Pex18 accumulates in Δpex4 and Δpex1 mutants, which might indicate that the turnover of Pex18 is associated with its normal function rather than abortive degradation [92]. Also in *Y. lipolytica*, interference with polyubiquitination or removal causes accumulation of Pex20 (a Pex18 orthologue) in peroxisomes [93].

The similarities in ubiquitination of the Pex5 and the Pex7/ Pex20 receptor complex are striking [93]. In the absence of either Pex4, Pex1, or Pex6 also Pex20 is degraded after polyubiquitination at residue K19 [93]. This position can be aligned to K21 in Pex5 [76]. Thus, it seems likely that ubiquitination of Pex5 and Pex20 family members plays a similar role in peroxisome biogenesis.

In *S. cerevisiae* wild-type cells, Pex5 has also been demonstrated to be monoubiquitinated. Monoubiquitininated Pex5 is localised to peroxisomes and requires the presence of functional docking and RING finger complexes [88], suggesting that it serves a non-degradative function that is a late event in peroxisomal matrix protein import. In search for an E2 for Pex5 ubiquitination, Pex4 is an obvious candidate. Monoubiquitininated Pex5 can only be detected in the presence of NEM, which might inhibit deubiquitinating enzymes [88], and therefore suggests that under wild-type conditions Pex5p is only transiently modified.

Based on the finding that the Pex5 level in a Δpex1 Δpex4 double deletion strain were reduced to that in a the Δpex1 single mutant strain, it was concluded, that Pex4 acts downstream of the AAA peroxins [13]. This proposal will need re-evaluation, if Pex4 turns out to provide the AAA substrates.

### 6. The peroxisomal RING complex

The three peroxins Pex2, Pex10, and Pex12 are conserved peroxisomal integral membrane proteins with RING domains in their cytoplasmically exposed C-termini. RING fingers bind zinc ions through their characteristic conserved cysteine and histidine residues. Zinc binding has been shown only for Pex10 [94], whereas part of the RING motifs in Pex2 and Pex12 are degenerate, so they might not coordinate zinc ions. The RING peroxins are required for peroxisome biogenesis in all species analysed [95–99]. A defect in PEX10 from Arabidopsis leads to a defective peroxisome and lipid droplet formation [100].

The RING peroxins interact with each other and with Pex5 [95,101–103]. Based on Pex5 stability in mutant strains, the RING complex peroxins have been placed downstream of the docking complex [13,101], fostering the idea of an import cascade, in which Pex5 is handed down from the docking complex to the RING complex [72]. The RING complex is itself associated with the docking complex through Pex8 [104]. Recently, it was shown that RING peroxins are required for Pex5 import in an in vitro system [85].

Pex5 ubiquitination has been reported to require the RING peroxins [86–88]. Thus, Pex22 might recruit the putative E2 Pex4 to the peroxisomal membrane and to the RING finger peroxins which might function as E3s in poly- and/or monoubiquitination of Pex5 or other substrates [72]. In line with this assumption, an interaction between Pex4 and Pex10 has been observed [105].
7. Functional role of AAA peroxins in Pex5 recycling

A ‘shuttling receptor model’ as proposed for the peroxisomal protein import process [80, 81] implies that receptor release is part of receptor cycling. Release is understood here as the removal of the unloaded cycling receptor from the peroxisomal membrane, a process that itself might be coupled to cargo release if the ‘transient pore hypothesis’ [60] holds true.

Experiments using a refined in vitro import system in which radiolabeled Pex5 is incubated with rat liver post-nuclear supernatants indicated that ATP is needed predominantly for the recycling of the import receptor at the end of the cycle rather than for its insertion into the membrane [84, 106]. Two different populations of Pex5 could be identified: the ‘stage 2’ form in the presence of ATP, in which Pex5 adopts the properties of a transmembrane protein with a short N-terminal domain accessible from the cytosolic side; and the ‘stage 3’ appearing under ATP-limiting conditions in which Pex5 is resistant to protease treatment [84]. It was further shown that stage 2 precedes stage 3 and that both Pex5 populations can be precipitated with anti-Pex14-antibodies [84]. Thus, binding and import of Pex5 seemingly does not need ATP hydrolysis, whereas the export of the receptor does [83–85]. Membrane-bound Pex5 is a target for an ATP-dependent component of the peroxisomal protein import machinery, which mediates its release from the membrane. This membrane associated chaperone machinery could be identified as the AAA peroxins complex [83, 85].

In yeast mutants with defective AAA peroxins or a deficiency in their membrane anchor Pex15, polyubiquitinated Pex5 accumulates at the peroxisomal membrane, while in cells affected in proteasomal degradation the polyubiquitinated species also appear in the cytosol [83]. These data support the idea that the polyubiquitinated Pex5-species are designated for proteasomal degradation. (Fig. 1; [83,86,88]. If, however, the AAA mutant and the proteasomal mutant are combined, polyubiquitinated Pex5 is again found exclusively in the membrane fraction where it is neither removed nor degraded. This indicates (1) that the release of polyubiquitinated Pex5 depends on the presence of the AAA peroxins and (2) that Pex5 polyubiquitination and AAA-dependent release are indeed sequential steps in a degradative pathway [83]. Finally, in an in vitro assay, the purified Pex1–Pex6-complex was demonstrated to function as a dislocase in the ATP-dependent removal of polyubiquitinated Pex5 from the peroxisomal membrane [83]. As these polyubiquitinated Pex5 species proved to be carbonate-resistant, these data indicated that the AAA peroxins perform the dislocation of polyubiquitinated integral Pex5 which then is directed to proteasomal disposal. However, the polyubiquitinated Pex5 species only represent a minor fraction of the total Pex5 at the peroxisomal membrane and it was demonstrated that also the non-ubiquitinated Pex5 pool is released from the membrane in an AAA peroxin and ATP-
dependent manner [83]. Thus, polyubiquitination does not seem to be a precondition for the removal of Pex5 from the peroxisomal membrane and it is tempting to speculate that the observed monoubiquitination of Pex5 might play a role as an export signal [83, 88] (Fig. 1). However, this remains to be investigated. When the localisation of the remaining Pex5 after the export assay was tested, most of the carbonate resistant Pex5 was removed from the membrane [83], indicating that membrane-integrated Pex5 is a major target for Pex1–Pex6-dependent export. In vitro studies with CHO cells also showed that ATP was not required for Pex5 import but was indispensable for its export [84,85]. Moreover, it was demonstrated that Pex5 was imported into the peroxisome remnants of cells that were defective in Pex1, Pex6, or Pex26 in an ATP-independent manner but that these cells were defective in Pex5 release, thereby providing evidence that the AAA peroxins and Pex26 are essential for the Pex5 export/release from the peroxisome to the cytosol also in higher eukaryotes [85]. Evidence for a role of Pex6 in Pex5 recycling has also been found in Arabidopsis, where a pex6 mutant has reduced levels of PEX5, and defects in PEX6 can be partially rescued by overexpression of PEX5 [91].

Taken together, the published data clearly show that Pex5 is mono- and polyubiquitinated at the peroxisomal membrane and dislocated to the cytosol by the AAA peroxins in an ATP-dependent manner. In the cytosol, the released receptor is either degraded by the proteasome as in the case for the polyubiquitinated forms or made available for another round of import as part of the receptor cycle (Fig. 1). A major remaining question is the nature of the signal by which the membrane-bound import receptor is recognised by the AAA-peroxins. As outlined above, it might well be that Pex4-dependent monoubiquitination of Pex5 generates the recognition signal. Though there is no conclusive evidence for a direct interaction between the import receptor and the AAA peroxins, the N-terminal region of Pex5 is predestined for recognition by the AAA peroxins. This region proved to be required for the ATP-dependent recycling [107] and has recently shown to be unstructured [108]. We speculate that, in analogy to Cdc48 [65], the AAA peroxins recognise the unstructured N-terminus of Pex5 in a reaction that is specified by ubiquitination and possibly with the help of ubiquitin-binding adaptor proteins.

8. Conclusions and perspectives

A model is emerging for the recycling of Pex5 in which ubiquitination of Pex5 and its recognition by the AAA complex play a central role. In this model, peroxisomal matrix proteins are recognised by cytosolic import receptors, which direct them to a docking complex at the peroxisomal membrane. Upon association of cargo-loaded receptors with docking complex subunits, the receptors might insert into the peroxisomal membrane. A cascade of protein–protein interactions at the peroxisomal membrane leads to (mono-)ubiquitination and AAA peroxin-dependent release of the now unloaded receptor from the peroxisomal membrane to the cytosol, where the receptor becomes available for further rounds of matrix protein import (Fig. 1). Part of the receptor is polyubiquitinated, recognised and released by the AAA peroxins and degraded by the proteasome. It is likely that this double function in Pex5 fate is regulated by adaptors interacting with the AAA peroxins. It is also likely that the process of AAA dependent receptor release can also happen in the absence of ubiquitination, albeit at lower efficiency. Ubiquitination of Pex5 might facilitate the recruitment of the AAA machinery, possibly together with ubiquitin binding proteins as adaptors, or structural changes induced on Pex5 by ubiquitination might facilitate recognition by the AAA complex. These possibilities would bear resemblance to ERAD, where Cdc48 can directly recognise unstructured proteins, but needs adaptors to do so in vivo. The analogy to ERAD is indeed striking [109,110] and it can be expected that more similarities will be found in future.

In recent years, much has been learned on Pex5 ubiquitination and its peroxisomal release to the cytosol by the AAA peroxins. We wish to conclude by mentioning six areas for future investigation:

(1) To fully appreciate the role of Pex5 release in the process of matrix protein import, it will be necessary to understand the function of Pex5 in matrix protein import. Is Pex5 a cycling receptor that crosses the peroxisomal membrane, as suggested by the extended receptor shuttle model? Can Pex5 functionally integrate into lipid membranes, as has been suggested by the transient pore hypothesis?

(2) To better understand the release of Pex5, we will have to advance the in vitro reconstitution of Pex5 import and release by purified recombinant proteins with the goal of developing a cell- and ultimately cytosol-free system of Pex5 receptor release.

(3) The E2 enzymes for Pex5 ubiquitination are now coming into focus. The respective E3 enzymes are still elusive. The three peroxisomal RING-finger proteins are obvious candidates for E3s for peroxisomal receptor ubiquitination. It will be necessary to analyse their role and to elucidate whether they are involved in mono- and/or polyubiquitination of the import receptors.

(4) The suggestion of a link between Pex5 ubiquitination and Pex5 release by the AAA peroxins is compelling, yet there is no direct evidence for such an association. Knowledge of more factors involved in receptor release, for instance by the identification of a functional ubiquitin-binding protein or a deubiquitinating enzyme that interacts with the AAA peroxins would support the idea of an ubiquitin-dependent Pex5 recognition by the AAA peroxins.

(5) Another goal for the future is the elucidation of the role of receptor ubiquitination and possible AAA-peroxins-dependent recycling in the PTS2-dependent protein import pathway.

(6) The elucidation of how receptor membrane integration, ubiquitination and release are mechanistically integrated into the translocation of folded proteins across the peroxisomal membrane is one of the fascinating challenges for future research.
Acknowledgements

We are grateful to Wolfgang Girzalsky, Harald W. Platta and Wolfgang Schliebs, for reading of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (Em178/2-4, SFB642), the FP6 European Union Project ‘Peroxisome’ (LSHG-CT-2004-512018) and by the Fonds der Chemischen Industrie.

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Please cite this article as: Sven Thoms, Ralf Erdmann, Peroxisomal matrix protein receptor ubiquitination and recycling, Biochimica et Biophysica Acta (2006), doi:10.1016/j.bbamcr.2006.08.046


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Please cite this article as: Sven Thoms, Ralf Erdmann, Peroxisomal matrix protein receptor ubiquitination and recycling, Biochimica et Biophysica Acta (2006), doi:10.1016/j.bbamcr.2006.08.046