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Review

New insights into dynamic and functional assembly of the AAA peroxins, Pex1p and Pex6p, and their membrane receptor Pex26p in shuttling of PTS1-receptor Pex5p during peroxisome biogenesis ☆

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ABSTRACT

Peroxisome is a single-membrane organelle in eukaryotes. The functional importance of peroxisomes in humans is highlighted by peroxisome-deficient peroxisome biogenesis disorders such as Zellweger syndrome. Two AAA peroxins, Pex1p and Pex6p, are encoded by *PEX1* and *PEX6*, the causal genes for PBDs of complementation groups 1 and 4, respectively. *PEX26* responsible for peroxisome biogenesis disorders of complementation group 8 codes for C-tail-anchored type-II membrane peroxin Pex26p, the recruiter of Pex1p–Pex6p complexes to peroxisomes. Pex1p is targeted to peroxisomes in a manner dependent on ATP hydrolysis, while Pex6p targeting requires ATP but not its hydrolysis. Pex1p and Pex6p are most likely regulated in their peroxisomal localization onto Pex26p via conformational changes by ATPase cycle. Pex5p is the cytosolic receptor for peroxisome matrix proteins with peroxisome targeting signal type-1 and shuttles between the cytosol and peroxisomes. AAA peroxins are involved in the export from peroxisomes of Pex5p. Pex5p is ubiquitinated at the conserved cysteine11 in a form associated with peroxisomes. Pex5p with a mutation of the cysteine11 to alanine, termed Pex5p-C11A, abrogates peroxisomal import of proteins harboring peroxisome targeting signals 1 and 2 in wild-type cells. Pex5p-C11A is imported into peroxisomes but not exported, hence suggesting an essential role of the cysteine residue in the export of Pex5p. This article is part of a Special Issue entitled: AAA ATPases: structure and function.

1. Introduction

Peroxisomes are single-membrane-bounded organelles present in nearly all of eukaryotic cells. The functional consequence of peroxisomes in humans is emphasized by peroxisome biogenesis disorders (PBDs) including Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease, and rhizomelic chondrodysplasia punctata, of which the primary cause is the impaired biogenesis of peroxisomes [1,2]. Genetic heterogeneity consisting of 13 complementation groups (CGs) has been identified in PBDs [2–4] (Table 1). Human *PEX1* and *PEX6* responsible for PBDs of CG1 and CG4 were cloned by functional complementation assay using Chinese hamster ovary (CHO) cell mutants [5–7] and by expressed sequence tag homology search using yeast *PEX1* and *PEX6* [8–10]. Search for the pathogenic PBD genes was accomplished by functional cloning of *PEX26* responsible for CG8 PBDs [3,11]. Pex26p is a functional homologue of yeast Pex15p, but we found no detectable amino acid sequence similarity [12].

Peroxisomal matrix proteins are synthesized on free polyribosomes and post-translationally imported into peroxisomes [13]. According to the PTS1-receptor recycling model [14–19], cargo-loaded Pex5p targets peroxisomes, translocates across the peroxisomal membrane, unloads the cargoes, and finally exits back to the cytosol. This protein import process involves many peroxins including Pex14p, Pex13p, Pex2p, Pex10p, Pex12p, Pex26p, Pex1p, and Pex6p. Mechanisms and functional roles of the components at each step of the whole processes remain elusive. Pex1p and Pex6p are members of the large AAA-protein family involved in a wide range of different cellular processes including vesicular transport, DNA repair, proteolysis, and mitochondrial functions [20–22]. One common functional feature of the AAA proteins is protein folding or unfolding in an ATP-dependent manner. AAA proteins share one or two AAA-cassettes that is characterized by a conserved sequence of 200–250 amino acids, termed D1 or D2 domain, including the Walker A and B motifs for ATP-binding and ATP-hydrolysis, respectively [23,24]. Pex26p recruits Pex1p–Pex6p complexes to peroxisomes [3,25].

As a step toward understanding the function of Pex1p, Pex6p, and Pex26p in peroxisome biogenesis, we investigated mechanisms

Abbreviations: CG, complementation group; CHO, Chinese hamster ovary; NSF, N-ethylmaleimide-sensitive fusion protein; PBDs, peroxisome biogenesis disorders; PTS1 and PTS2, peroxisome targeting signal types 1 and 2; VCP, valocin-containing protein.

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Table 1
Complementation groups (CG) and PEX genes of peroxisome deficiencies.

Gene	CG		Phenotype	CHO mutants	Peroxisome ghosts	Peroxin	
	US/EU	Japan				(kDa)	Characteristics
PEX1	1	E	ZS, NALD*, IRD*	Z21, ZP107	+	143	AAA family
PEX2	10	F	ZS, IRD*	Z65	+	35	PMP, RING
PEX3	12	G	ZS	ZPG208	-	42	PMP
PEX5	2		ZS, NALD	ZP105*, ZP139	+	68	PTS1 receptor, TPR family
PEX6	4 (6)	C	ZS, NALD*	ZP92	+	104	AAA family
PEX7	11	R	RCDP	ZPG207	+	36	PTS2 receptor, WD motif
PEX10	7 (5)	B	ZS,	NALD	+	37	PMP, RING
PEX12	3		ZS, NALD, IRD	ZP109	+	40	PMP, RING
PEX13	13	H	ZS, NALD*	ZP128	+	44	PMP, PTS1-P, SH3
PEX14	15	K	ZS	ZP110	+	41	PMP, PTS-DP, PTS2-DP
PEX16	9	D	ZS		-	39	PMP
PEX19	14	J	ZS	ZP119	-	33	CAAX motif
PEX26	8	A	ZS, NALD*, IRD*	ZP124, ZP167	+	34	PMP, Pex1p–Pex6p recruiter

ZS, Zellweger syndrome; IRD, infantile Refsum disease; NALD, neonatal adrenoleukodystrophy; RCDP, rhizomelic chondrodysplasia punctata; PMP, peroxisome membrane protein; TPR, tetratricopeptide repeat.
* Temperature-sensitive phenotype.

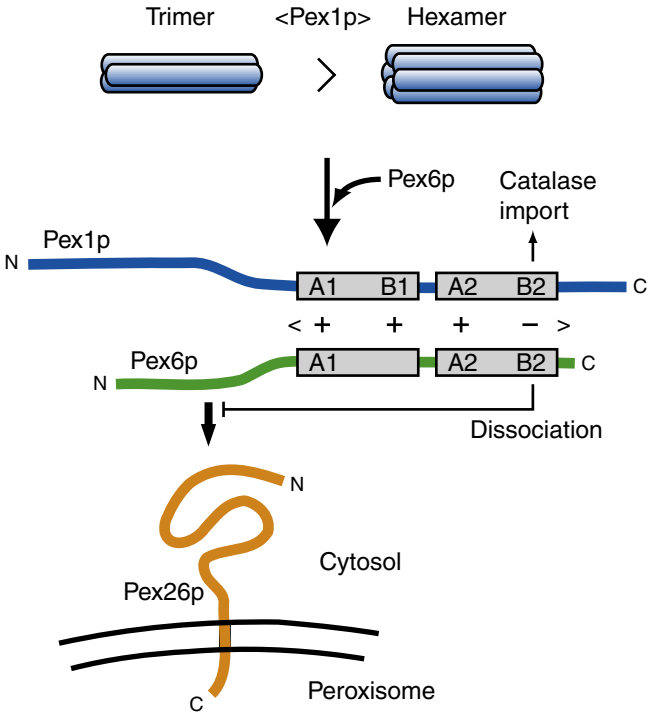

Fig. 1. A schematic model for Pex1p–Pex6p interaction and ternary complexes assembly with Pex26p.Cytosolic Pex1p is present mostly in a homo-trimer and partly in a hexamer. Pex1p oligomer is assembled to oligomer complexes with Pex6p. Walker motifs (A1, B1, and A2) of the AAA domain are essential for the interaction between Pex1p and Pex6p and their localization to peroxisomes (+), while the Walker B2 motif is dispensable (−). In contrast, ATP hydrolysis by Pex1p Walker B2 motif is required for catalase import, and that by Pex6p Walker B2 motif initiates the release of Pex6p from Pex26p.

underlying recruiting of Pex1p and Pex6p to Pex26p on peroxisome membrane. Pex1p and Pex6p are most likely regulated in their peroxisomal localization onto Pex26p via conformational changes by ATPase cycle. Peroxisome-targeting signal type 1 (PTS1)-receptor Pex5p is imported to and exported from peroxisomes [17,18], in ATP-independent and -dependent manners, respectively [17]. Mono-ubiquitination at a cysteine residue of Pex5p is required for the export from peroxisomes [26–28]. Moreover, Pex1p, Pex6p, and Pex26p are critical for the Pex5p export. This step seems to be responsible for the overall energy requirement of the import process, which gives rise to the export-driven import model [29]. This concept proposes that the ATP-dependent dislocation of the Pex5p by Pex1p and Pex6p is coupled to protein translocation into the organelle. We here address the Pex5p shuttling between peroxisomes and the cytosol.

2. Dynamic and functional assembly of the AAA peroxins, Pex1p and Pex6p, and their membrane receptor Pex26p

We identified the regions involved in the interaction of human Pex1p and Pex6p [30] and showed that two AAA-cassette structures (D1 and D2) and ATP-binding to the two AAA-cassettes of Pex1p and Pex6p are required for their association (Fig. 1). In *Saccharomyces cerevisiae*, the interaction of Pex1p and Pex6p involves the respective first AAA-cassette, requiring ATP-binding but not ATP-hydrolysis in the second AAA-cassette (D2) of Pex1p [12]. In contrast to this yeast system, the interaction of human Pex1p and Pex6p requires ATP binding to both D1 and D2 domains, suggesting that the interaction is enhanced by conformational changes in both D1 and D2 domains upon ATP binding [30].

We demonstrated the importance of Walker motifs A1, B1 and A2 in Pex1p, and Walker motifs A1 and A2 in Pex6p, for their peroxisomal localization, in good agreement with the finding of Pex1p–Pex6p heterooligomer in binding assays [30]. Neither Pex1p nor Pex6p is localized to peroxisomes in the absence of its mutual partner, thereby suggesting that the localization of Pex1p and Pex6p on peroxisomes requires the formation of the ternary complexes with Pex26p *in vivo*. It is also noteworthy that Pex6p likely interacts with Pex26p in the absence of Pex1p [3,30,31]. Pex1p may stabilize Pex6p by forming the ternary complex on peroxisomes. It is also possible that Pex1p and Pex6p may interact with other peroxins that regulate the subcellular localization and function of AAA peroxins.

Pex1p shows dynamic conformational changes in the presence or absence of Pex6p and Pex26p [30]. Pex1p forms two distinct oligomeric structures on peroxisomal membrane or in the cytoplasm. As depicted by Blue-Native PAGE analysis of cytosolic Pex1p from HEK293 cells, Pex1p is mostly in a homo-trimer, and less in a homo-hexamer.

Crystal structure of the N-terminal domain of mouse Pex1p resembles valosin-containing protein (VCP/p97), another member of the AAA proteins [32,33]. VCP/p97 forms a barrel-like homohexameric structure [34]. VCP/p97 as well as Pex1p possesses a highly protease-sensitive C-terminal domain, which is conversely protected from tryptic digestion upon nucleotide-binding in the case of VCP/p97 [35]. Thus, it is plausible that Pex1p forms a hexameric ring structure upon ATP binding and undergoes a structural change during ATPase cycle in a manner similar to VCP/p97 (Fig. 2). Likewise, ATP-binding to Pex6p may trigger a conformational change and regulate its homo-oligomerization.

Pex1p and Pex6p are involved in early and late steps of peroxisome biogenesis including membrane fusion [36] and protein dislocation such as Pex5p export from peroxisomes [17,18,37]. The yeast Pex1p and Pex6p likewise play pivotal roles in peroxisome biogenesis [18,38–40]. These functions of Pex1p and Pex6p are reminiscent of those of N-ethylmaleimide-sensitive fusion protein (NSF) and VCP/p97. AAA ATPases play various roles including vesicle fusion by NSF [41,42] and ER-associated protein degradation by VCP/p97/CDC48 [43]. Despite their main localization in the cytosol, NSF and VCP/p97 exert their functions on membranes in vesicle fusion [44,45] and ER-associated protein degradation [46], respectively. Pex1p and