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Minireview

Peroxisome biogenesis and peroxisome biogenesis disorders

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Abstract Peroxisome assembly in mammals requires more than 15 genes. Two isoforms of the peroxisome targeting signal type 1 (PTS1) receptor, Pex5pS and Pex5pL, are identified in mammals. Pex5pS and Pex5pL bind PTS1 proteins. Pex5pL, but not Pex5pS, directly interacts with the PTS2 receptor, Pex7p, carrying its cargo PTS2 protein in the cytosol. Pex5p carrying the cargos, PTS1 and PTS2, docks with the initial site Pex14p in a putative import machinery, subsequently translocating to other components such as Pex13p, Pex2p, Pex10p and Pex12p, whereby the matrix proteins are imported. The peroxins, Pex3p, Pex16p and Pex19p, function in the assembly of peroxisomal membrane vesicles that precedes the import of matrix proteins. Hence, peroxisomes may form de novo and do not have to arise from pre-existing, morphologically recognizable peroxisomes. Impaired peroxisome assembly causes peroxisome biogenesis disorders such as Zellweger syndrome.

Key words: Peroxin; Chinese hamster ovary cell mutant; Peroxisomal protein import; Membrane assembly; Zellweger syndrome; Pathogenic gene

1. Introduction

Peroxisomes are present in a wide variety of eukaryotic cells, from yeast to humans, and they function in various metabolic pathways, including the β-oxidation of very long chain fatty acids and the synthesis of ether-lipids [1]. Peroxisomal proteins, including membrane proteins, are encoded by nuclear genes and are translated on free polyribosomes in the cytosol [2]. Peroxisomes are thought to be formed by the division of pre-existing peroxisomes after the import of newly synthesized proteins [2]. Genetic analyses of peroxisome biogenesis-defective mutants of yeast and mammalian cells have led to the identification of a number of protein factors, termed peroxins, which are essential for peroxisome assembly [3–7]. The functional consequence of human peroxisomes is highlighted by fatal genetic peroxisome biogenesis disorders (PBDs), including Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease and rhizomelic chondrodysplasia puntata, all of which are linked to a failure of peroxisome biogenesis [4–8]. Genetic heterogeneity comprising more than 15 complementation groups (CGs) has been identified in mammals, by CG analysis using fibroblasts from patients with PBD and peroxisome-deficient Chinese hamster ovary (CHO) cell mutants [9,10]. Therefore, more than 15 genes are likely to be involved in mammalian peroxisome biogenesis.

Mechanisms of peroxisome assembly, including peroxisomal import of newly synthesized proteins, are one of the major foci in peroxisome research. Studies on both peroxisome biogenesis and PBDs at the molecular level are rapidly progressing. The identification and characterization of numerous genes that are essential for peroxisome biogenesis, by means of the genetic phenotype-complementation of peroxisome assembly-defective mutants of mammalian somatic cells such as CHO cells and of several yeast species including *Saccharomyces cerevisiae*, *Pichia pastoris*, *Hansenula polymorpha* and *Yarrowia lipolytica*, have made invaluable contributions to the study of peroxisome biogenesis [4–7] and protein trafficking in eukaryotes [11]. I herein summarize recent findings on peroxisome biogenesis factors involved in matrix protein import and membrane assembly in mammals and pathogenic genes responsible for PBDs.

2. Genetic approaches to studying mammalian peroxisome biogenesis

Two mutually distinct but complementary approaches have been taken in order to isolate mammalian peroxin genes.

2.1. Isolation of peroxisome-deficient mammalian cell mutants

Two methods have been developed for the isolation of mammalian somatic cell mutants defective in peroxisome biogenesis: (i) colony autoradiographic screening with a phenotypic marker, dihydroxyacetonephosphate acyltransferase deficiency [12,13] and (ii) the photo-sensitized selection method using 9-(1′-pyrene)nonanol and an exposure to long wavelength ultraviolet (UV) light [14]. Using these two methods, we have so far isolated 13 CGs of peroxisome-deficient CHO cell mutants [4,10,15–17]. All CHO cell mutants showed a phenotype of deficiency in peroxisome biogenesis, as noted in fibroblasts from PBD patients [13,18,19]. Peroxisomal remnants, called membrane ghosts, are seen in most CHO mutants [4], except for ZP119 [15] and ZPG208 [10], as reported for fibroblasts from peroxisome-deficient PBD patients [20]. A complete set of CG analyses by cell fusion between 13 CGs of CHO cell mutants and 13 CGs of fibroblasts from patients with PBDs revealed that nine CGs of CHO mutants represent the human PBD CGs (Table 1) [10,15–18]. CHO mutants, ZP110, ZP114, ZP126 and ZPG208, were distinct from any

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Abbreviations: CG, complementation group; CHO, Chinese hamster ovary; EST, expressed sequence tag; PBD, peroxisome biogenesis disorder; PTS, peroxisomal targeting signal

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of the 13 human CGs. Accordingly, peroxisome assembly apparently requires at least 17 gene products in mammals, including humans.

2.2. Genes required for peroxisome biogenesis

2.2.1. Genetic phenotype-complementation assay. Nine peroxin cDNAs, *PEX1*, *PEX2* (formerly PAF-1), *PEX3*, *PEX5*, *PEX6*, *PEX12*, *PEX13*, *PEX14* and *PEX19*, have been cloned by functional phenotype-complementation assay on CHO cell mutants [17,21–29] (Fig. 1). We also showed most of these *PEX*s to be responsible for human PBDs [17,21,24,26,27,29–32] (Table 1).

2.2.2. Expressed sequence tag (EST) homology search. An alternative strategy, i.e. the homology search by screening the EST database using yeast *PEX* genes, has successfully led to the isolation of human orthologue genes responsible for PBDs: *PEX1* [33,34], *PEX5* [35], *PEX6* [36], *PEX7* [37–39], *PEX10* [40,41], *PEX12* [42] and *PEX16* [43,44].

3. Biogenesis of peroxisomes

3.1. Import of matrix proteins and import machinery

The import of most peroxisomal matrix proteins is mediated by two types of *cis*-acting peroxisomal targeting signals (PTSs): the C-terminal uncleavable tripeptide PTS1, -S/A/C-K/R/H-L/(M) [45,46], and the nonapeptide presequence PTS2, -(R/K)(L/V/I)X_5(H/Q)(L/A)-, located at the N-terminus [47,48]. *PEX5* and *PEX7* encode the receptors for PTS1 and PTS2, respectively. A deficiency of Pex5p, a member of the tetratricopeptide repeat family, causes PBDs of CG2 manifesting protein import defects [24,35,49] (Table 1). Dysfunction of Pex7p is responsible for CG11 RCDP showing a cell phenotype of impaired PTS2 import [37–39].

Two phenotypically distinct groups of *PEX5*-defective CG2 CHO cell mutants were isolated [24]. One group of *pex5* CHO mutants such as ZP105 showed the import defect of both PTS1 and PTS2 proteins, whilst another group represented by ZP139 was impaired in the transport of PTS1 proteins but not of PTS2 proteins, exactly as in yeast *pex5* mutants. Fibroblasts with such distinct phenotypes from patients of CG2 were also identified [35,50]. In mammals, including the Chinese hamster and humans, two isoforms of Pex5p termed Pex5pS and Pex5pL with an internal 37 amino acid insertion have been identified [24,50], whilst in yeast only a single type of Pex5p was isolated [5,6]. Pex5pS and Pex5pL form a homomeric as well as heteromeric dimer [51]. The expression of either type of Pex5p complemented the impaired PTS1 import in CHO *pex5* mutants as in yeast, but only Pex5pL could rescue the PTS2 import defect noted in a subgroup such as ZP105 [24]. Likewise, only Pex5pL can restore PTS2 import in fibroblasts from a CG2 patient with Zellweger syndrome [50]. We recently found that Pex5pL directly interacts with the PTS2 receptor, Pex7p, carrying its cargo PTS2 protein in the cytosol [51,52]. Pex5pL, but not Pex5pS, translocates the Pex7p and PTS2 protein complex to peroxisomes. Furthermore, a recently isolated *pex5* mutant cell line, ZPG231, showed a novel phenotype, *PEX5*-defective but impaired solely in the PTS2 import [52]. The missense point mutation, Ser214Phe, in the *PEX5* gene disrupted the interaction of Pex5pL with Pex7p, resulting in complete elimination of the PTS2 import pathway. Therefore, it is evident that Pex5pL plays an exclusively pivotal role in PTS2 transport, in addition to PTS1 import.

Mammalian *PEX14* encoding a 41 kDa protein was isolated by functional complementation of a CHO mutant, ZP110 [28] or by an EST search using a yeast gene [53,54]. Pex14p was characterized as an integral membrane protein of peroxisomes, exposing its N- and C-terminal parts to the cytosol [28]. Pex5p is localized mostly in the cytosol in the wild-type CHO-K1 and Pex14p-deficient mutant cells, whilst it accumulates to the peroxisomal remnants in cell mutants defective of Pex13p and the RING family peroxins such as Pex2p and Pex12p [51]. Furthermore, overexpression of Pex14p [28], but not Pex10p [40], Pex12p [27] or Pex13p [17], causes accumulation of Pex5p to peroxisomal membranes, with concomitant interference with the PTS1 and PTS2 import [51]. Therefore, the import of PTS1 and PTS2 possibly shares the common, if not exclusive, translocon, as inferred from the common phenotype, the impaired import of both PTS1 and PTS2, in mammalian cell mutants, *pex2*, *pex10*, *pex12*, *pex13*

Table 1
CGs and complementing genes of peroxisome deficiency

Fibroblasts		Phenotype[a]	CHO mutants	Complementing gene
Japan	USA/Europe			
A	8	ZS, NALD, IRD	ZP124	
B	7 (5)	ZS, NALD		*PEX10*
C	4	ZS, NALD	ZP92	*PEX6*
D	9	ZS		*PEX16*
E	7	ZS, NALD, IRD	Z24, ZP107	*PEX1*
F	10	ZS, IRD	Z65	*PEX2* (PAF-1)
	2	ZS, NALD	ZP105, ZP139	*PEX5*
	3	ZS	ZP109	*PEX12*
	6	ZS, NALD		
G		ZS		
H		ZS, NALD	ZP128	*PEX13*
J		ZS	ZP119	*PEX19*
R	11	RCDP	ZPG207	*PEX7*
			ZP110	*PEX14*
			ZP114	
			ZP126	
			ZPG208	*PEX3*

[a]ZS, Zellweger syndrome; NALD, neonatal adrenoleukodystrophy; IRD, infantile Refsum disease; RCDP, rhizomelic chondrodysplasia punctata.

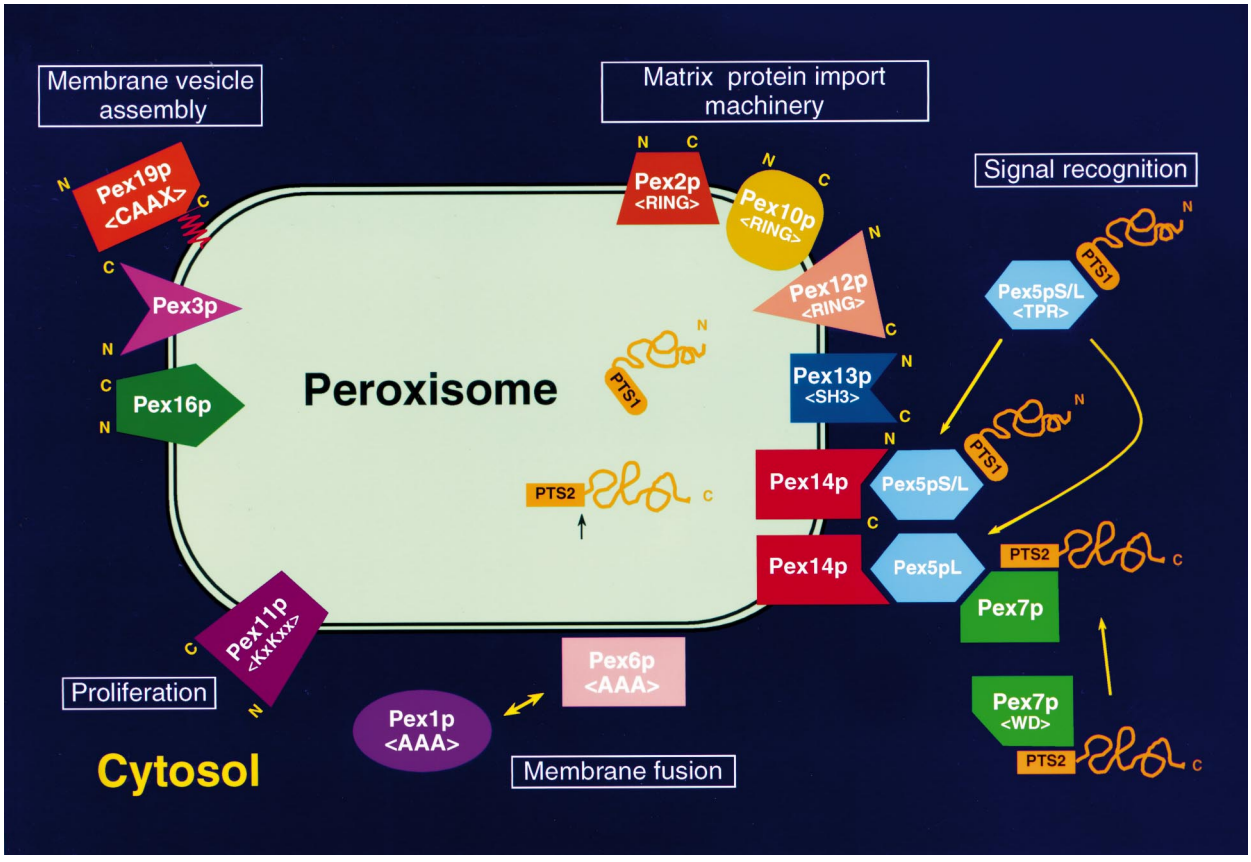

Fig. 1. A schematic view of peroxisome biogenesis in mammals. The intracellular locations and molecular properties of peroxins so far identified are shown. Peroxins are divided into four groups: (i) peroxins that are required for matrix protein import; (ii) those including Pex3p, Pex16p and Pex19p, responsible for peroxisome membrane assembly; (iii) those such as Pex1p and Pex6p of the AAA family presumably involved in membrane fusion step and (iv) those such as Pex11p apparently involved in proliferation. Import of matrix proteins has been better understood: matrix proteins, PTS1 and PTS2 proteins, are recognized by Pex5p and Pex7p, respectively. Two isoforms of Pex5p are identified in mammals. PTS1 proteins are transported by Pex5pS and Pex5pL to peroxisomes, where Pex14p functions as a convergent component of 'protein import machinery' (for details, see text). Pex5pL directly interacts with the PTS2 receptor, Pex7p, carrying its cargo PTS2 protein in the cytosol and translocates the Pex7p-PTS2 protein complex to the initial docking site Pex14p. Pex5p carrying the cargos subsequently translocates to other components such as Pex13p, Pex2p, Pex10p and Pex12p. PTS1 and PTS2 proteins are then released at the inner surface and/or inside of peroxisomes. Both Pex5p and Pex7p finally shuttle back to the cytosol.

and *pex14*. Collectively, these findings demonstrate that a mobile shuttle signal-receptor Pex5p, carrying the cargos PTS1 as well as Pex7p-PTS2 proteins, most likely docks with the initial site Pex14p in a potential machinery for the import of matrix proteins and subsequently translocates to other machinery-components such as Pex13p, Pex2p, Pex10p and Pex12p (Fig. 1). After unloading the cargos, Pex5p and Pex7p, in a bound form or independently, cycle back to the cytosol. In contrast to the mammalian system, in yeast Pex5p-PTS1 proteins and Pex7p-PTS2 polypeptides independently bind to Pex14p [55].

3.2. Membrane assembly

Three mammalian peroxins, Pex3p, Pex16p and Pex19p, have been isolated either by functional phenotype-complementation assay on CHO cell mutants [23,29] or by the EST database search using yeast *PEX* genes [43,44,56,57] and shown to be essential for peroxisome membrane assembly.

3.2.1. Pex19p. *PEX19* encodes a farnesylated protein partially, if not completely, anchored to peroxisome membranes [29,58]. Pex19p is identical to the previously isolated PxF [59]. *HsPEX19* expression complemented impaired peroxisome biogenesis in fibroblasts from a patient with CG-J PBD [29]. This patient possessed a homozygous, inactivating mutation: a one-base insertion, ^{764}A, in a codon for ^{255}Met resulted in a frameshift, inducing a 24 amino acid sequence entirely distinct from normal Pex19p. Upon transfection of *HsPEX19* into a CHO *pex19* mutant ZP119 devoid of peroxisomal 'ghosts' [15], the most striking was the formation of peroxisomal membranes followed by the import of matrix proteins [29]. This was the first observation of the membrane assembly process during peroxisome biogenesis, particularly differentiated from the import of soluble proteins.

3.2.2. Pex16p. *HsPEX16* encoding a 336 amino acid peroxisomal protein was isolated by the EST homology search using *Y. lipolytica PEX16* [43,44]. Fibroblasts from a Zellweger patient of CG-D (the same group as CG9 in USA) are defective in peroxisomal membrane biogenesis and morphologically devoid of peroxisomal remnants, as in *PEX19*-defective fibroblasts of CG-J [9,29]. *HsPEX16* expression restored peroxisomal membrane assembly and matrix protein import in CG-D fibroblasts [43]. Membrane biogenesis apparently precedes the import of soluble proteins [44] (Honsho and Fujiki, unpublished observation). The mutation, Arg176Ter, in *PEX16* was identified as the genetic cause of CG-D PBD.

3.3.3. Pex3p. Pex3p expression re-established peroxisome

formation in *pex3* mutants from CHO cells [10,23] and yeasts, *H. polymorpha* [60] and *P. pastoris* [61], all absent from peroxisomal membrane structures. Upon expression of *PEX3* in a CHO *pex3* mutant ZPG208, peroxisomal membrane vesicles were assembled prior to the import of soluble proteins such as PTS1 and PTS2 proteins [23], as in *pex19* [29] and *pex16* [43,44] cell mutants, implying the temporally differentiated translocation of matrix proteins into peroxisomal membrane vesicles. Pex3p was characterized as an integral membrane protein of peroxisomes, exposing its N- and C-terminal parts to the cytosol [23]. A homozygous, inactivating missense mutation, G to A at position 413 in a codon (GGA) for ^{138}Gly resulting in a codon (GAA) for Glu, was the genetic cause of peroxisome deficiency of *pex3* ZPG208 [23]. The peroxisome-restoring activity apparently required the full length of Pex3p, while its N-terminal part from residues 1 to 40 was sufficient to target a fusion protein to peroxisomes [23].

Collectively, Pex3p, Pex16p and Pex19p can be categorized as peroxins essential for the assembly of peroxisome membranes. They may function as essential factors required for the translocation process of membrane proteins and/or membrane vesicle assembly, possibly in a concerted manner. Pex3p indeed interacts with Pex19p, both in vivo and in vitro [23], as recently found in *S. cerevisiae* [58] and *P. pastoris* [62], as well as in human cells [57]. Pex3p may function in peroxisomal membrane assembly at an early stage by interacting with Pex19p but not with Pex16p. However, it is not clear whether or not Pex3p is a prerequisite peroxin for Pex19p to be localized to and/or anchored on peroxisomal membranes. Pex3p does not appear to require Pex19p for targeting in mammals [23] and may function upstream of Pex19p as noted in *P. pastoris* [62]. It is also noteworthy that Pex3p and Pex19p were very recently suggested to play a role in recognizing newly synthesized membrane proteins and transporting them to 'preperoxisome' vesicles [63,64]. Taken together, peroxisomes may form de novo and do not have to arise from pre-existing, morphologically recognizable peroxisomes. At such an early stage of peroxisome assembly the endoplasmic reticulum (ER) may be involved, as was suggested for Pex2p and Pex16p, both initially residing in ER in *Y. lipolytica* [65]. However, no direct evidence for the involvement of ER in peroxisome assembly has been noted in mammalian cells. The assembled membrane vesicles then import other membrane components, including those of a potential matrix protein import machinery such as Pex14p, Pex13p and the RING family peroxins, to form 'premature peroxisomes' which are capable of importing matrix proteins. The matured peroxisomes finally divide so that progeny peroxisomes emerge.

4. Perspective

More than 15 CGs of mammalian cell mutants have been identified, including PBD patients' fibroblasts and CHO mutant cell lines [10]. Pathogenic genes have been elucidated for most of the PBD CGs and only a few are as yet unidentified. The biochemical functions of several peroxins involved in the import of matrix proteins have been better elucidated. However, the molecular mechanisms by which most of the peroxins function in peroxisome biogenesis, particularly in membrane assembly, remain to be addressed. Future investigations using the cloned peroxins and *pex* mutants including *PEX* gene-knock-out mice [66,67], will shed light on the mechanisms involved in peroxisome biogenesis and the pathogenesis of PBD.

5. Note

I apologize for not citing all invaluable papers recently contributed owing to the limitation of space.

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