



Peroxisome biogenesis in mammalian cells

Yukio Fujiki*, Kanji Okumoto, Satoru Mukai, Masanori Honsho and Shigehiko Tamura

Department of Biology, Faculty of Sciences, Kyushu University Graduate School, Fukuoka, Japan

Edited by:

Vladimir I. Titorenko, Concordia University, Canada

Reviewed by:

Yasuyoshi Sakai, Kyoto University, Japan

Vladimir I. Titorenko, Concordia University, Canada

*Correspondence:

Yukio Fujiki, Department of Biology, Faculty of Sciences, Kyushu University Graduate School, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
e-mail: yfujiki@kyudai.jp

To investigate peroxisome assembly and human peroxisome biogenesis disorders (PBDs) such as Zellweger syndrome, thirteen different complementation groups (CGs) of Chinese hamster ovary (CHO) cell mutants defective in peroxisome biogenesis have been isolated and established as a model research system. Successful gene-cloning studies by a forward genetic approach utilized a rapid functional complementation assay of CHO cell mutants led to isolation of human peroxin (*PEX*) genes. Search for pathogenic genes responsible for PBDs of all 14 CGs is now completed together with the homology search by screening the human expressed sequence tag database using yeast *PEX* genes. Peroxins are divided into three groups: (1) peroxins including Pex3p, Pex16p, and Pex19p, are responsible for peroxisome membrane biogenesis via classes I and II pathways; (2) peroxins that function in matrix protein import; (3) those such as three forms of Pex11p, Pex11p α , Pex11p β , and Pex11p γ , are involved in peroxisome proliferation where DLP1, Mff, and Fis1 coordinately function. In membrane assembly, Pex19p forms complexes in the cytosol with newly synthesized PMPs including Pex16p and transports them to the receptor Pex3p, whereby peroxisomal membrane is formed (Class I pathway). Pex19p likewise forms a complex with newly made Pex3p and translocates it to the Pex3p receptor, Pex16p (Class II pathway). In matrix protein import, newly synthesized proteins harboring peroxisome targeting signal type 1 or 2 are recognized by Pex5p or Pex7p in the cytoplasm and are imported to peroxisomes via translocation machinery. In regard to peroxisome-cytoplasmic shuttling of Pex5p, Pex5p initially targets to an 800-kDa docking complex consisting of Pex14p and Pex13p and then translocates to a 500-kDa RING translocation complex. At the terminal step, Pex1p and Pex6p of the AAA family mediate the export of Pex5p, where Cys-ubiquitination of Pex5p is essential for the Pex5p exit.

Keywords: CHO cell mutants, genetic phenotype-complementation, import machinery, membrane assembly, pathogenic genes, peroxins, peroxisome targeting signals, Zellweger syndrome

INTRODUCTION

Molecular mechanisms of peroxisome biogenesis, including peroxisomal import of newly synthesized matrix and membrane proteins, have been one of the major foci in the peroxisome research. Studies at the molecular level on both peroxisome assembly and peroxisome biogenesis disorders (PBDs) rapidly progressed in the last three decades. Studies on cloning of genes, particularly including those of a very low-level expression, have benefited from so-called functional cloning of genes, mostly cDNAs in mammalian cases, by phenotype complementation assay using cell mutants defective of biological pathways. The identification and characterization of numerous essential genes, termed *PEX*s encoding peroxisome biogenesis factors termed peroxins, by means of the genetic phenotype-complementation of peroxisome assembly-defective cell mutants, named *pex* mutants impaired in *PEX* genes. Such mutants from Chinese hamster ovary (CHO) cells (Table 1; see below) (Fujiki, 1997, 2000), several yeast species including *Saccharomyces cerevisiae* (Erdmann

et al., 1989), *Pichia pastoris* (Gould et al., 1992; Liu et al., 1992), *Hansenula polymorpha* (Cregg et al., 1990), and *Yarrowia lipolytica* (Nuttley et al., 1993) (also see reviews Van Der Klei and Veenhuis, 1996; Kunau, 1998; Tabak et al., 1999; Subramani et al., 2000; Titorenko and Rachubinski, 2001; Lazarow, 2003), and plant *Arabidopsis thaliana* (Hayashi and Nishimura, 2006) have made invaluable contributions to the investigations of peroxisome biogenesis and protein trafficking in eukaryotes (Schatz and Dobberstein, 1996; Wickner and Schekman, 2005). We herein summarize mammalian model cell systems in studying biogenesis, physiology, and human disorders of peroxisomes.

GENETIC APPROACHES TO STUDYING MAMMALIAN PEROXISOME BIOGENESIS

Basically two mutually complementary approaches have been taken for isolation of *PEX* genes encoding peroxins, i.e., the genetic phenotype-complementation of peroxisome biogenesis-defective mutants of mammalian somatic cells such as CHO cells and a combination of the human ortholog isolation by homology search on the human expressed sequence tag (EST) database using yeast *PEX* genes and cells derived from the patients with PBDs of 14 different genotypes, i.e., complementation groups (CGs)

Abbreviations: CG, complementation group; CHO, Chinese hamster ovary; DLP1, dynamin-like protein 1; EST, expressed sequence tag; PBD, peroxisome biogenesis disorder; PTS, peroxisomal targeting signal; ZS, Zellweger syndrome.

(Table 1; see below) (Fujiki, 1997, 2000, 2003; Gould and Valle, 2000; Weller et al., 2003).

MAMMALIAN CELL MUTANTS DEFICIENT OF PEROXISOME

Genetic heterogeneity consisting of 14 CGs were identified in PBDs by cell-fusion CG analysis using fibroblast cell lines derived from PBD patients (Fujiki, 2000; Ghaedi et al., 2000a; Gould and Valle, 2000; Matsumoto et al., 2001), where CGs 4 and 7 were revealed to be the same CGs as CGs 6 and 5, respectively (Table 1). A new CG, CG15, of ZS was also identified (Shimozawa et al., 2004), hence indicative of totally 13 genotypes of PBDs. The primary defect for PBDs was revealed to be the impaired biogenesis of peroxisomes (Fujiki, 2000; Gould and Valle, 2000). With respect to somatic animal cell mutants, 12 CGs of peroxisome-deficient CHO cell mutants were isolated, including a mutant ZP114 of a CG distinct from human CGs (Figure 1; Table 1). A PBD patient of the 14th CG, CG16, was recently identified with pathogenic gene *PEX11β* (Ebberink et al., 2012). Together, genetic heterogeneity comprising 15 CGs are currently identified in mammals including humans and CHO cells.

PEROXISOME BIOGENESIS GENES

Genetic phenotype-complementation screening

*PEX*s were isolated by genetic phenotype complementation of peroxisome biogenesis-deficient mutants of mammalian somatic cells including CHO cells (Figure 1A) and of several yeast species including *S. cerevisiae*, *P. pastoris*, *H. polymorpha*, and *Y. lipolytica* (Distel et al., 1996; Subramani et al., 2000; Fujiki et al., 2006b). Two mutually distinct but complementary approaches have been taken to identify and clone mammalian *PEX* genes.

A direct cloning approach has been taken by means of genetic complementation with peroxin cDNA essential for assembly of

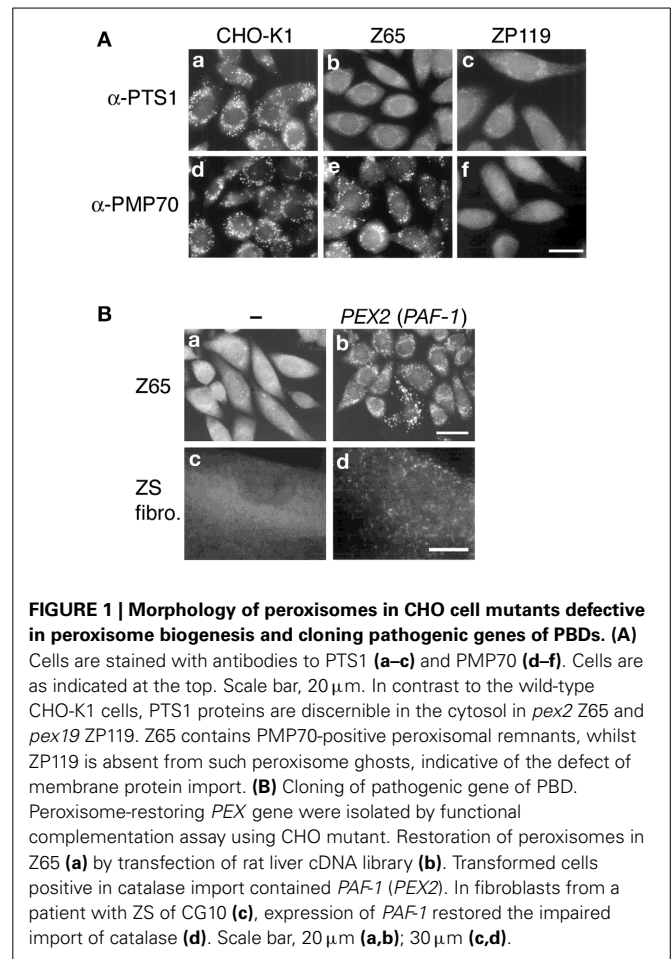


Table 1 | Complementation groups (CGs) and *PEX* genes of peroxisome deficiencies.

Gene	CG		Phenotype	CHO mutants	Peroxisome ghosts	Peroxin	
	US/EU	Japan				(kDa)	Characteristics
<i>PEX1</i>	1	E	ZS, NALD*, IRD*	Z24, ZP107	+	143	AAA family
<i>PEX2</i>	10	F	ZS, IRD*	Z65	+	35	PMP, RING
<i>PEX3</i>	12	G	ZS	ZPG208	–	42	PMP, PMP-DP
<i>PEX5</i>	2		ZS, NALD	ZP105*, ZP139	+	68	PTS1 receptor, TPR family
<i>PEX6</i>	4(6)	C	ZS, NALD*	ZP92	+	104	AAA family
<i>PEX7</i>	11	R	RCDP	ZPG207	+	36	PTS2 receptor, WD motif
<i>PEX10</i>	7(5)	B	ZS, NALD		+	37	PMP, RING
<i>PEX11β</i>	16		ZS		+	28	PMP
<i>PEX12</i>	3		ZS, NALD, IRD	ZP109	+	40	PMP, RING
<i>PEX13</i>	13	H	ZS, NALD*	ZP128	+	44	PMP, PTS1-DP, SH3
<i>PEX14</i>	15	K	ZS	ZP110	+	41	PMP, PTS1-DP, PTS2-DP
<i>PEX16</i>	9	D	ZS		–	39	PMP, PMP-DP
<i>PEX19</i>	14	J	ZS	ZP119	–	33	CAAX motif, PMP receptor
<i>PEX26</i>	8	A	ZS, NALD*, IRD*	ZP124, ZP167 ZP114	+	34	PMP, Pex1p-Pex6p recruiter

*, Temperature-sensitive phenotype.

ZS, Zellweger syndrome; IRD, infantile Refsum disease; NALD, neonatal adrenoleukodystrophy; RCDP, rhizomelic chondrodysplasia punctata; DP, docking protein; PMP, peroxisome membrane protein; TPR, tetratricopeptide repeat.

peroxisomes in CHO cells. Establishment of an effective method, termed P12 (12-(1'-pyrene)dodecanoic acid)/ultraviolet selection method, made it feasible to isolate revertant (transfectant) cells showing a morphologically and biochemically normal peroxisome-phenotype, whereby *PEX2* (formerly *PAF-1*) encoding the 35-kDa membrane peroxin Pex2p with RING zinc-finger motif was cloned for the first time (Tsukamoto et al., 1991) (Figure 1B). Expression of *PEX2* (called *Zellweger gene*) in fibroblasts from a ZS patient of CG10 (F) complemented the impaired peroxisome biogenesis (Shimozawa et al., 1992) (Figure 1B). Dysfunction of *PEX2* caused by a homozygous nonsense point mutation at R119ter was shown for the first time to be responsible for ZS, a prototype of the PBDs (Shimozawa et al., 1992). A more practical approach, i.e., a transient expression assay, was also developed for further isolation of *PEX* cDNAs including nine others, *PEX1*, *PEX3*, *PEX5*, *PEX6*, *PEX12*, *PEX13*, *PEX14*, *PEX19*, and *PEX26* (Fujiki, 2003; Fujiki et al., 2006b) (Figure 2). These *PEX*s were shown to be the pathogenic genes involved in PBDs of nine CGs (Weller et al., 2003; Fujiki et al., 2006b; Fujiki, 2011) (Table 1).

Expressed sequence tag homology search

As an alternative method, the homology search by screening the human EST database using yeast *PEX* genes successfully led to isolation of human ortholog genes responsible for PBDs: *PEX1*, *PEX3*, *PEX5*, *PEX6*, *PEX7*, *PEX10*, *PEX12*, *PEX13*, and *PEX16* (Weller et al., 2003; Fujiki et al., 2006b).

All of pathogenic genes responsible for PBDs of currently identified 13 CGs have been successfully cloned within about 10 years after the first isolation of the ZS gene, *PEX2*, by such extensive search using the mutually complementary methods.

BIOGENESIS OF PEROXISOMES

MEMBRANE BIOGENESIS

Three mammalian peroxins, Pex3p, Pex16p, and Pex19p, were isolated by the functional phenotype-complementation assay on CHO cell mutants (Matsuzono et al., 1999; Ghaedi et al., 2000b) and the EST database search using yeast *PEX* genes (Kammerer et al., 1997, 1998; Honsho et al., 1998; South and Gould, 1999) and were shown to be exclusively required for membrane

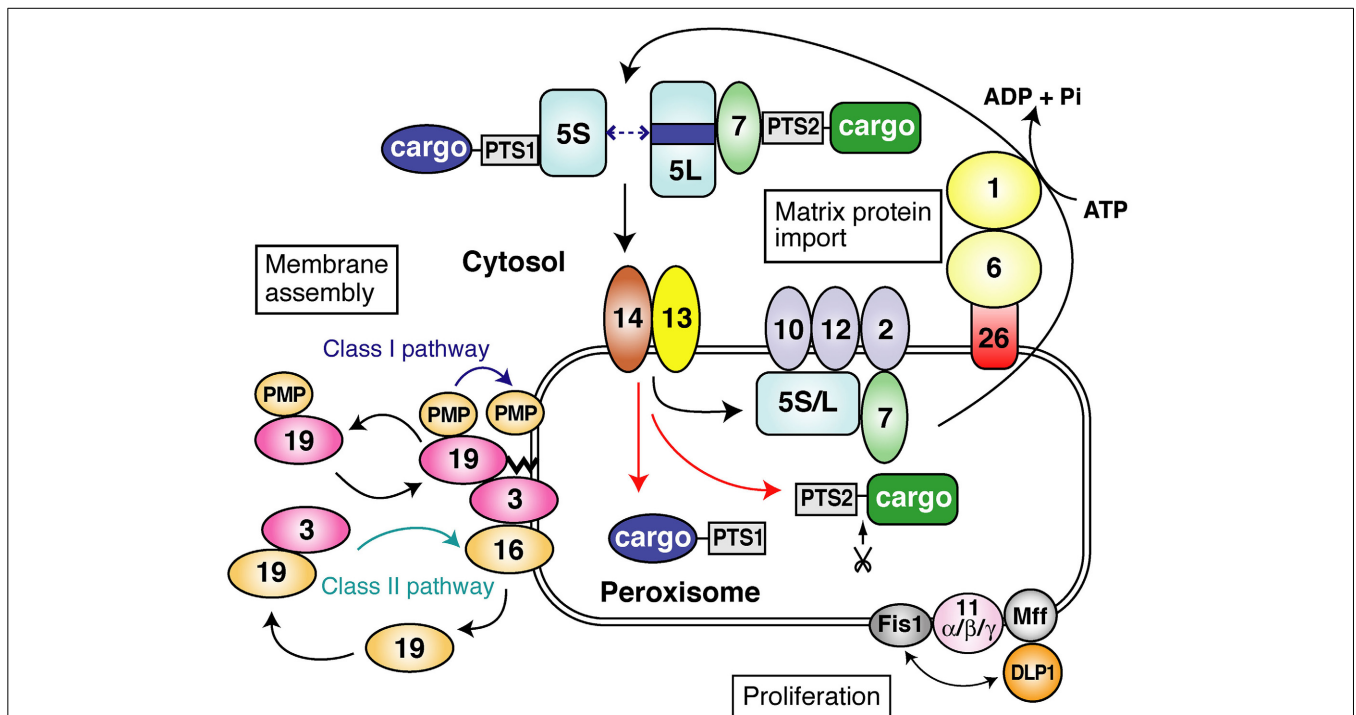


FIGURE 2 | A schematic view of peroxisome biogenesis in mammalian cells. The subcellular localization and molecular characteristics of peroxins are shown. Peroxins are classified into three groups: (1) peroxins that are required for matrix protein import; (2) those including Pex3p, Pex16p and Pex19p, responsible for peroxisome membrane assembly via classes I and II pathways (see in this figure); (3) those such as three forms of Pex11p, Pex11 α , Pex11 β , and Pex11 γ , apparently involved in peroxisome proliferation where DLP1, Mff, and Fis1 coordinately function. PTS1 and PTS2 proteins are recognized by Pex5p and Pex7p, respectively, in the cytoplasm. Two isoforms, Pex5pS and Pex5pL, of Pex5p are identified in mammals. PTS1 proteins are transported by homo- and hetero-oligomers of Pex5pS and Pex5pL to peroxisomes, where Pex14p functions as a convergent, initial docking site of the “protein import machinery” translocon. Pex5pL directly

interacts with the PTS2 receptor, Pex7p, carrying its cargo PTS2 protein in the cytosol and translocates the Pex7p–PTS2 protein complex to Pex14p. PTS1 and PTS2 proteins are then released at the inner surface and/or inside of peroxisomes, downstream Pex14p and upstream Pex13p. Pex5p and Pex7p subsequently translocate to other translocon components, named translocation complex comprising the RING peroxins, Pex2p, Pex10p, and Pex12p. Both Pex5p and Pex7p finally shuttle back to the cytosol. In regard to peroxisome-cytoplasmic shuttling of Pex5p, Pex5p initially targets to an 800-kDa docking complex containing Pex14p and then translocates to a 500-kDa translocation complex comprising RING peroxins. At the terminal step of the protein import reaction, Pex1p and Pex6p of the AAA family catalyze the export of Pex5p, where Cys-ubiquitination of Pex5p is prerequisite to the Pex5p exit.

assembly of peroxisomes. Mechanistic insights on membrane biogenesis are addressed here.

Peroxis essential for membrane assembly of peroxisomes

Of 13 peroxins of which mutations are responsible for PBDs, Pex3p, Pex16p, and Pex19p were identified as essential factors for PMP assembly in several species including humans (Baerends et al., 1996; Götte et al., 1998; Honsho et al., 1998; Matsuzono et al., 1999; South and Gould, 1999; Ghaedi et al., 2000a; Hettema et al., 2000; Sacksteder et al., 2000; South et al., 2000; Otzen et al., 2004) (**Figure 1**). Pex19p is a predominantly cytoplasmic protein that shows a broad PMP-binding specificity; Pex3p serves as the membrane-anchoring site for Pex19p-PMP complexes (Class I pathway); and Pex16p—a protein absent in most yeasts (Eitzen et al., 1997; South and Gould, 1999) functions as the receptor for Pex19p complexes with newly synthesized Pex3p (Matsuzaki and Fujiki, 2008) (Class II pathway) (**Figures 2, 3**). The function of Pex16p is not conserved between different species. In addition, under debate remains whether Pex19p has a chaperone-like role in the cytosol or at the peroxisome membrane and/or functions as a cycling import receptor for newly synthesized PMPs (Fujiki et al., 2006a).

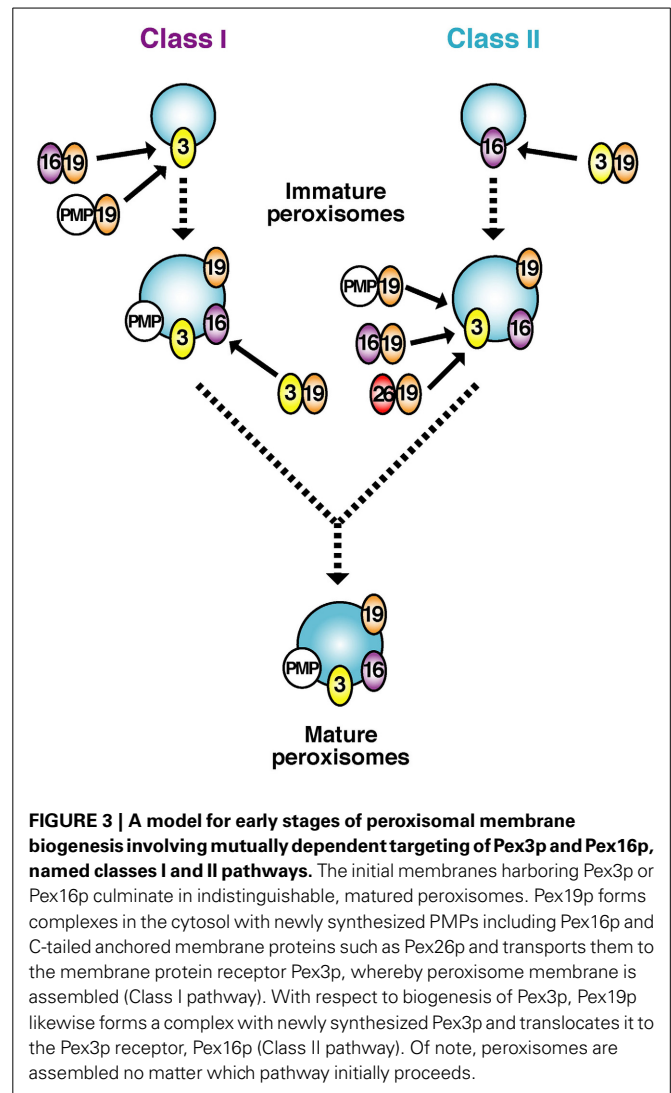
Gene defects of peroxins required for both membrane biogenesis and matrix protein import

Impairment of Pex3p, Pex16p, and Pex19p, causes the most severe PBD, ZS, of three CGs, CG12 (G), CG9 (D), and CG14 (J), respectively (Weller et al., 2003; Fujiki et al., 2006b, 2012; Fujiki, 2011) (**Table 1**).

Pex19p. *PEX19* encodes 33-kDa farnesylated protein harboring farnesylation CAAX box motif localized mostly in the cytosol and only partly anchored to peroxisomal membranes (Matsuzono et al., 1999). *PEX19* expression complemented impaired peroxisome assembly in fibroblasts from a patient with CG14 (J) PBD. This patient was a homozygote for inactivating mutation: a one-base insertion, A764, in a codon for Met²⁵⁵, thereby resulting in a frameshift. Upon transfection of *PEX19* into a CHO *pex19* mutant ZP119 devoid of peroxisomal remnants called ghosts, most striking was formation of peroxisomal membranes, prior to the import of matrix proteins (Matsuzono et al., 1999; Sacksteder et al., 2000). This was the first demonstration of the membrane assembly process during peroxisome biogenesis, particularly differentiated from the soluble protein import.

Pex16p. Fibroblasts from a ZS patient of CG9 (D) are deficient in peroxisomal membrane remnants, as in *PEX19*-defective fibroblasts of CG14 (J). Expression of *PEX16* encoding 336-amino-acid peroxisomal membrane protein restored peroxisomal membrane biogenesis and matrix protein import in CG9 (D) fibroblasts (Honsho et al., 1998; South and Gould, 1999), of which mutation was a homozygous nonsense mutation R176ter (Honsho et al., 1998). More recently, other mutations are identified: exon 10 skip (Shimozawa et al., 2002) and five novel homozygous mutations (Ebberink et al., 2010).

Pex3p. Mammalian *PEX3* encodes 42-kDa integral membrane protein of peroxisomes (Ghaedi et al., 2000a,b). Upon expression



of *PEX3* in a CHO *pex3* mutant (Ghaedi et al., 2000b) and fibroblasts from three ZS patients of CG12 (G) (Ghaedi et al., 2000a), peroxisomal membrane vesicles were assembled prior to the import of soluble proteins (Ghaedi et al., 2000a; Muntau et al., 2000; Shimozawa et al., 2000; South et al., 2000; Fujiki et al., 2006a; Fujiki, 2011), as in *pex19* and *pex16* patients-derived cells (see above), likewise implying the temporally differentiated translocation of matrix proteins into peroxisomal membrane vesicles. Two types of mutations, exon 11 deletion and a single-nucleotide insertion in the codon for Val¹⁸² in exon 7, in *PEX3* were identified in the ZS patients (Ghaedi et al., 2000a; Muntau et al., 2000; Shimozawa et al., 2000).

Taken together, Pex3p, Pex16p, and Pex19p are categorized as a peroxin exclusively required for the assembly of peroxisome membranes. They function as essential factors in the transport process of membrane proteins and membrane vesicle assembly in a concerted manner. Two distinct pathways were recently suggested for the import of PMPs: a Pex19p- and Pex3p-dependent

class I pathway for PMP-import complex, except for Pex3p (Fang et al., 2004; Matsuzono et al., 2006) and a Pex19p- and Pex16p-dependent class II pathway for Pex3p (Matsuzaki and Fujiki, 2008) (Figures 2, 3). It is noteworthy that C-tailed anchor-type peroxin Pex26p, the recruiter of Pex1p-Pex6p complex, is transported in a Pex19p-dependent (Halbach et al., 2006), class I pathway (Yagita et al., 2013), which is distinct from the GET3-dependent topogenesis of yeast Pex15p, a functional ortholog of Pex26p (Schuldiner et al., 2008).

Involvement of ER in peroxisome biogenesis

In regard to involvement of the ER in peroxisome biogenesis, ER was postulated to provide the initial “seed” for recruiting other components required for peroxisome assembly (Kim et al., 2006; Ma et al., 2011; Tabak et al., 2013). Several groups suggested a different view of peroxisomal membrane biogenesis that peroxisomes are formed from ER upon induction of Pex3p (Hoepfner et al., 2005; Kragt et al., 2005; Kim et al., 2006), although the significance of such observations remains under debate. Recently, a study (Motley and Hettema, 2007) suggest that peroxisomes are generally formed by growth and division under normal conditions and that only under a condition where no peroxisome is present in a cell, they can be formed from the ER after the expression of the complementing *PEX* gene, whilst another study (Van Der Zand et al., 2010) proposes that all peroxisomal membrane proteins are transported via ER. Meanwhile, we demonstrated that Pex3p, the membrane receptor for Pex19p-complexes with PMPs including Pex16p, is directly targeted to peroxisomes in a Pex19p-Pex16p dependent class II pathway in mammalian cells such as CHO and human cell lines (Matsuzaki and Fujiki, 2008). Moreover, it is noteworthy that several peroxisomal membrane proteins might be transported to peroxisomes via ER (Lam et al., 2010; Agrawal et al., 2011; Yonekawa et al., 2011), likely implying a sort of semi-autonomous property of peroxisomes. At any event, the issue with respect to how peroxisome membrane is assembled is one of the important and of highly interesting problems to be tackled (Ma et al., 2011; Fujiki et al., 2012; Tabak et al., 2013).

MATRIX PROTEIN IMPORT

Ten peroxins including Pex1p, Pex2p, Pex5p, Pex6p, Pex7p, Pex10p, Pex12p, Pex13p, Pex14p, and Pex26p are involved in protein import into peroxisomal matrix (Figure 2) (Fujiki et al., 2006a).

Peroxisome-cytoplasmic shuttling of import receptors

PTS1 and PTS2 proteins are recognized by Pex5p and Pex7p, respectively, in the cytoplasm. In mammalian cells, PTS1 proteins are transported by homo- and hetero-oligomers of Pex5pS and Pex5pL to peroxisomes, where Pex14p functions as the initial site of an 800-kDa “docking complex.” Pex5pL translocates the Pex7p–PTS2 protein complex to Pex14p (Otera et al., 2002; Miyata and Fujiki, 2005). After releasing the cargoes, Pex5p and Pex7p translocate to a 500-kDa “translocation complex” comprising the RING peroxins, Pex2p, Pex10p and Pex12p (Miyata and Fujiki, 2005). Both Pex5p and Pex7p finally translocate back to the cytosol (Dammai and Subramani, 2001; Gouveia et al., 2003; Nair et al., 2004; Miyata and Fujiki, 2005; Platta et al.,

2005; Miyata et al., 2009). At the terminal step of the protein import reaction, AAA peroxins, Pex1p and Pex6p, recruited to Pex26p (Pex15p in yeast) on peroxisomes catalyze the ATP-dependent export of Pex5p (Miyata and Fujiki, 2005; Platta et al., 2005). Ubiquitination of Pex5p is prerequisite for the Pex5p exit (Carvalho et al., 2007; Williams et al., 2007; Okumoto et al., 2011).

Mono-ubiquitination of the conserved cysteine residue at position 11 in the N-terminal region of mammalian Pex5p plays an essential role in the recycling, especially in the export step from peroxisomes to the cytosol (Grou et al., 2009; Okumoto et al., 2011; Miyata et al., 2012), as in yeast (Platta et al., 2009). A cytosolic factor, AWP1/ZFAND6 involved in the recycling of Pex5p is recently identified in mammals (Miyata et al., 2012); USP9X and Ubp15 are suggested as a potential deubiquitinase in mammals (Grou et al., 2012) and yeast (Debelyy et al., 2011), respectively. A distinct redox state may affect the recycling of Pex5p requiring Cys-ubiquitination, thereby leading as a possible cause to the phenotype of deficiency in protein import in *PEX*-defective cells.

REDOX STATE OF NORMAL AND PEROXISOME-DEFICIENT CELLS

In peroxisomes possessing a fatty acid β -oxidation system in wild-type CHO cells, the redox state within the peroxisomes is more reductive than that in the cytosol, despite the fact that reactive oxygen species are generated within the peroxisomes (Yano et al., 2010). Moreover, to our surprise, the redox state in the cytosol of *pex* cell mutants is more reductive than that of the wild-type CHO cells (Yano et al., 2010). Such distinct redox state may affect the recycling of Pex5p requiring Cys-ubiquitination, thereby leading as a possible cause to the phenotype of deficiency in protein import in *PEX*-defective cells including cell lines from patients with PBDs. A potential way to cure the PBD patients may be a screening for agents that moderate the abnormal cytosolic redox state in the *pex* cell lines including the cells with nonredox-sensitive mutations in *PEX*s. It is noteworthy that in *P. pastoris* PTS1-cargo release from Pex5p is achieved by a redox-regulated oligomer to dimer transition of Pex5p and aided by Pex8p (Ma et al., 2013). Interestingly, intraperoxisomal redox status is strongly influenced by environmental growth conditions (Ivashchenko et al., 2011).

GENE DEFECTS OF PROTEINS FOR PEROXISOMAL MORPHOGENESIS

Three isoforms of Pex11p family, Pex11p α (Abe et al., 1998; Li et al., 2002a), Pex11p β (Abe and Fujiki, 1998; Schrader et al., 1998; Li et al., 2002b), and Pex11p γ (Li et al., 2002a; Tanaka et al., 2003), are identified as factors involved in morphogenesis of peroxisomes in mammals (Kobayashi et al., 2007; Delille et al., 2010; Koch et al., 2010; Itoyama et al., 2013). In mammalian cells, dynamin-like protein 1 (DLP1) (Koch et al., 2003; Li and Gould, 2003; Tanaka et al., 2006; Waterham et al., 2007), fission 1 (Fis1) (Koch et al., 2005; Kobayashi et al., 2007), and mitochondrial fission factor (Mff) (Gandre-Babbe and Van Der Bliet, 2008; Otera et al., 2010; Koch and Brocard, 2012; Itoyama et al., 2013) are shown to be involved in the fission of peroxisomes.

In regard to peroxisomal dysmorphogenesis in humans, only two patients have been identified with a different defect in any

of the proteins involved in the proliferation and division of peroxisomes. The first reported patient was a severely affected female patient, who died 1 month after birth and postmortally was found to have a dominant-negative heterozygous mutation in the *DLP1* gene, which resulted in a severe fission defect of both peroxisomes and mitochondria (Waterham et al., 2007). More recently, the first patient with a defect of peroxisomal division due to a homozygous nonsense mutation in the *PEX11β* gene was reported as the 14th CG (CG16) of PBDs (Ebberink et al., 2012) (Table 1).

PERSPECTIVE

Mammalian cell mutants of 15 CGs defective of peroxisome biogenesis have been identified, including PBD patients' fibroblasts and CHO mutant cell lines (Table 1). Pathogenic genes are now elucidated for all of PBD CGs. Biochemical functions of peroxins involved in the import of matrix proteins are better elucidated, whilst molecular mechanisms underlying the membrane assembly are less understood. Defects in peroxisomal morphogenesis have also been recently reported. Investigations using the cloned peroxins and *pex* mutants including CHO mutants and those from PBD patients will shed light on the mechanisms involved in biogenesis and morphogenesis of peroxisomes and pathogenesis of PBDs.

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