Review

Peroxisome biogenesis and human peroxisome-deficiency disorders

By Yukio FUJIKI[*1,†]

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Abstract: Peroxisome is a single-membrane-bounded ubiquitous organelle containing a hundred different enzymes that catalyze various metabolic pathways such as β-oxidation of very long-chain fatty acids and synthesis of plasmalogens. To investigate peroxisome biogenesis and human peroxisome biogenesis disorders (PBDs) including Zellweger syndrome, more than a dozen different complementation groups of Chinese hamster ovary (CHO) cell mutants impaired in peroxisome biogenesis are isolated as a model experimental system. By taking advantage of rapid functional complementation assay of the CHO cell mutants, successful cloning of *PEX* genes encoding peroxins required for peroxisome assembly invaluably contributed to the accomplishment of cloning of pathogenic genes responsible for PBDs. Peroxins are divided into three groups: 1) peroxins including Pex3p, Pex16p and Pex19p, are responsible for peroxisome membrane biogenesis via Pex19p- and Pex3p-dependent class I and Pex19p- and Pex16p-dependent class II pathways; 2) peroxins that function in matrix protein import; 3) those such as Pex11pβ are involved in peroxisome division where DLP1, Mff, and Fis1 coordinately function.

Keywords: CHO cell mutants, peroxisome biogenesis, protein import machinery, pathogenic genes, peroxins, Zellweger syndrome

1. Introduction

Peroxisomes are single-membrane-bounded ubiquitous organelles containing a hundred different enzymes that catalyze various metabolic pathways, including β-oxidation of very long-chain fatty acids, the synthesis of ether lipids such as plasmalogens, and bile-acid metabolism[1] (Table 1). They were discovered in 1954, named peroxisomes in 1965, and defined that peroxisomes contain one or more enzymes that use molecular oxygen to remove hydrogen atoms and form hydrogen peroxide from organic substrates.[2] Catalase, a typical marker enzyme of peroxisomal matrix, degrades hydrogen peroxides.

Peroxisomes are thought to form by the division of pre-exiting peroxisomes after the import of newly synthesized proteins.[3] Molecular mechanisms of peroxisome biogenesis, including peroxisomal import of newly synthesized matrix and membrane proteins, have been extensively investigated basically by several eukaryotic systems. Studies at the molecular level on both peroxisome assembly and peroxisome biogenesis disorders (PBDs, Table 2) rapidly progressed in the last three to four decades. The identification and isolation of over 30 essential genes termed *PEX*s encoding peroxisome biogenesis factors named peroxins, have been performed by means of the genetic phenotype-complementation of peroxisome assembly-deficient cell mutants, named *pex* mutants impaired in *PEX* genes. Such mutants from Chinese hamster ovary (CHO) cells (Table 3; see below),[4),5] several yeast species including *Saccharomyces cerevisiae*,[6] *Pichia pastoris*,[7),8] *Hansenula polymorpha*,[9] and *Yarrowia lipolytica*[10] (also see reviews[11)–16]), and plant *Arabidopsis thaliana*[17] have been successfully contributing to the investigations

*1 Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.
† Correspondence should be addressed: Y. Fujiki, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan (e-mail: yfujiki@kyudai.jp).

Abbreviations: CG: complementation group; CHO: Chinese hamster ovary; DHAP-ATase: dihydroxyacetonephosphate acyltransferase; DLP1: dynamin-like protein 1; IRD: infantile Refsum disease; NALD: neonatal adrenoleukodystrophy; PBD: peroxisome biogenesis disorder; PMP: peroxisomal integral membrane protein; PTS: peroxisome-targeting signal; RCDP: rhizomelic chondrodysplasia punctata; ZS: Zellweger syndrome.

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Table 1. Functions of peroxisome[a]

1.	Respiration: H_2O_2-producing oxidases, catalase
2.	Fatty acid β-oxidation: acyl-CoA oxidase, bifunctional protein (hydratase-dehydrogenase), thiolase
3.	Ether glycerolipid (plasmalogen) biosynthesis: DHAP-ATPase, alkyl-DHAP synthase
4.	Transamination and oxidation (gluconeogenesis): serine-pyruvate aminotransferase (alanine-glyoxylate aminotransferase)
5.	Purine catabolism
6.	Polyamine catabolism
7.	Bile acid biosynthesis
8.	Pipecolic acid catabolism
9.	Phytanic acid catabolism

[a]Representative functions in mammalian peroxisomes are listed, where peroxisomal enzymes involved in the functions (1 to 4) are also described.

Table 2. Peroxisomal disease

Peroxisome biogenesis disorders (PBDs)
Zellweger spectrum disorders
Zellweger syndrome (ZS)
Neonatal adrenoleukodystrophy (NALD)
Infantile Refsum disease (IRD)
Rhizomelic chondrodysplasia punctata (RCDP)
Single-enzyme deficiencies
Adrenoleukodystrophy (ALD)
Acyl-CoA oxidase deficiency
D-Bifunctional protein deficiency
3-Ketoacyl-CoA thiolase deficiency
Refsum disease (phytanyl-CoA hydroxylase deficiency), α-Methylacyl-CoA racemase deficiency
Hyperoxaluria type I (alanine glyoxylate aminotransferase deficiency)
Mevalonate kinase deficiency
Glutaric aciduria 3 (glutaryl-CoA oxidase deficiency)
Acatalasemia

of peroxisome biogenesis and protein traffics in eukaryotes.[18),19)] I herein review and address peroxisome biogenesis and human deficiency disorders by making use of mammalian model cell systems.

2. Genetic approaches to studying mammalian peroxisome biogenesis

Two mutually complementary genetic approaches used for isolation of *PEX* genes encoding peroxins were genetic phenotype-complementation of peroxisome assembly-defective mutants of CHO cells and a combination of the human orthologue isolation by homology search on the human expressed sequence tag database using yeast *PEX* genes and cells from patients with PBDs of more than a dozen different genotypes, *i.e.*, complementation groups (CGs) (Table 3; see below).[4),5),20)–22)]

2.1. Mammalian cell mutants deficient of peroxisome.

2.1.1. Cell lines from patients with PBDs. The PBDs include Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), infantile Refsum disease (IRD), which are called Zellweger syndrome spectrum, and rhizomelic chondrodysplasia punctata (RCDP)[23),24)] (Table 2). Patients with ZS show severe neurological abnormalities, characteristic dysmorphism and hepatomegaly, and rarely survive with an average life of only 6 months. NALD patients have the symptoms similar to ZS patients, but they survive a little longer, early childhood. By contrast, patients with IRD do not manifest significant abnormalities in the central nervous system, and survive with the longest average life, 3–11 years.[23)] RCDP patients show distinct phenotypic character-

Table 3. Complementation groups (CGs) and *PEX* genes of peroxisome deficiencies

Gene	CG		PBD	CHO mutants	Ps-memb. biogenesis[a]	Peroxin	
	US/EU	Japan				(kDa)	Characteristics
PEX1	1	E	ZS, NALD*, IRD*	Z24, ZP107	+	143	AAA family
PEX2	10	F	ZS, IRD*	Z65	+	35	PMP, RING
PEX3	12	G	ZS	ZPG208	−	42	PMP, PMP-DP
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
PEX11β	16		ZS		+	28	PMP
PEX12	3		ZS, NALD, IRD	ZP109	+	40	PMP, RING
PEX13	13	H	ZS, NALD*	ZP128	+	44	PMP, PTS1-DP, SH3
PEX14	15	K	ZS	ZP110	+	41	PMP, PTS1-DP, PTS2-DP
PEX16	9	D	ZS		−	39	PMP, PMP-DP
PEX19	14	J	ZS	ZP119	−	33	CAAX motif, PMP receptor
PEX26	8	A	ZS, NALD*, IRD*	ZP124, ZP167	+	34	PMP, Pex1p-Pex6p recruiter
				ZP114	+		

*, temperature-sensitive phenotype. [a]Peroxisomal membrane assembly is normal (+) or impaired (−).
PBD, peroxisomal biogenesis disorders; ZS, Zellweger syndrome; IRD, infantile Refsum disease; NALD, neonatal adrenoleukodystrophy; RCDP, rhizomelic chondrodysplasia punctata; DP, docking protein; PMP, peroxisome membrane protein; TPR, tetratricopeptide repeat.

istics such as severe growth failure and rhizomelia. Genetic heterogeneity consisting of 14 CGs has been identified in PBDs by cell-fusion CG analysis using fibroblast cell lines derived from PBD patients[5),20),25)–27)] (Table 3), where the primary cause for PBDs was revealed to be the impaired biogenesis of peroxisomes.[5),20)]

2.1.2. Isolation of CHO cell lines. Two methods were developed for the isolation of mammalian somatic cell mutants defective in peroxisome biogenesis: (i) colony autoradiographic screening with a phenotypic marker, dihydroxyacetonephosphate acyltransferase (DHAP-ATase) deficiency;[28),29)] and (ii) the photo-sensitized selection method using 9-(1′-pyrene)nonanol (P9OH) and an exposure to long wave-length ultraviolet (UV) light which kills wild-type cells incorporating P9OH as a fatty alcohol into plasmalogens and survive cell mutants deficient in such activity.[30),31)] We so far isolated 12 CGs of peroxisome-deficient CHO cell mutants by these methods (Table 3). All of CHO cell mutants showed a typical phenotype of deficiency in peroxisome biogenesis, such as the impaired protein import, no catalase latency, severely affected DHAP-ATase activity, as noted in fibroblasts from PBD patients.[5),29)]

A complete set of CG analyses by cell-fusion between 12 CGs of CHO cell mutants and 13 CGs of fibroblasts from patients with PBDs revealed that 11 CGs of CHO mutants represent the human PBD CGs[5),29)] (Table 3). A PBD patient of the 14th CG, CG16, was recently identified.[32)] Together, genetic heterogeneities comprising 15 CGs are currently reported in mammals including humans and CHO cells.

2.2. Peroxisome biogenesis genes.

2.2.1. Genetic phenotype-complementation screening. *PEX*s were isolated by genetic phenotype-complementation of peroxisome biogenesis-deficient mutants of mammalian somatic cells such as CHO cells (Fig. 1A) and of several species of yeast including *S. cerevisiae*, *P. pastoris*, *Y. lipolytica*, and *H. polymorpha*.[12),24),33),34)] Forward genetics method using animal somatic cell mutants such as CHO cell mutants was shown to be a highly effective approach for isolating essential genes including the peroxin genes, *PEX*s.

We searched for the gene encoding a factor complementing the impaired peroxisome biogenesis of one, Z65, of the CHO cell mutants by transfecting a rat liver cDNA library.[35)] Transfectants were selected by the 12-(1′-pyrene) dodecanoic acid (P12)/UV method,[36)] showing peroxisomes as verified by staining with anti-catalase antibody (Fig. 1B). An open reading frame encoded a novel 35-kDa peroxisomal integral membrane protein with

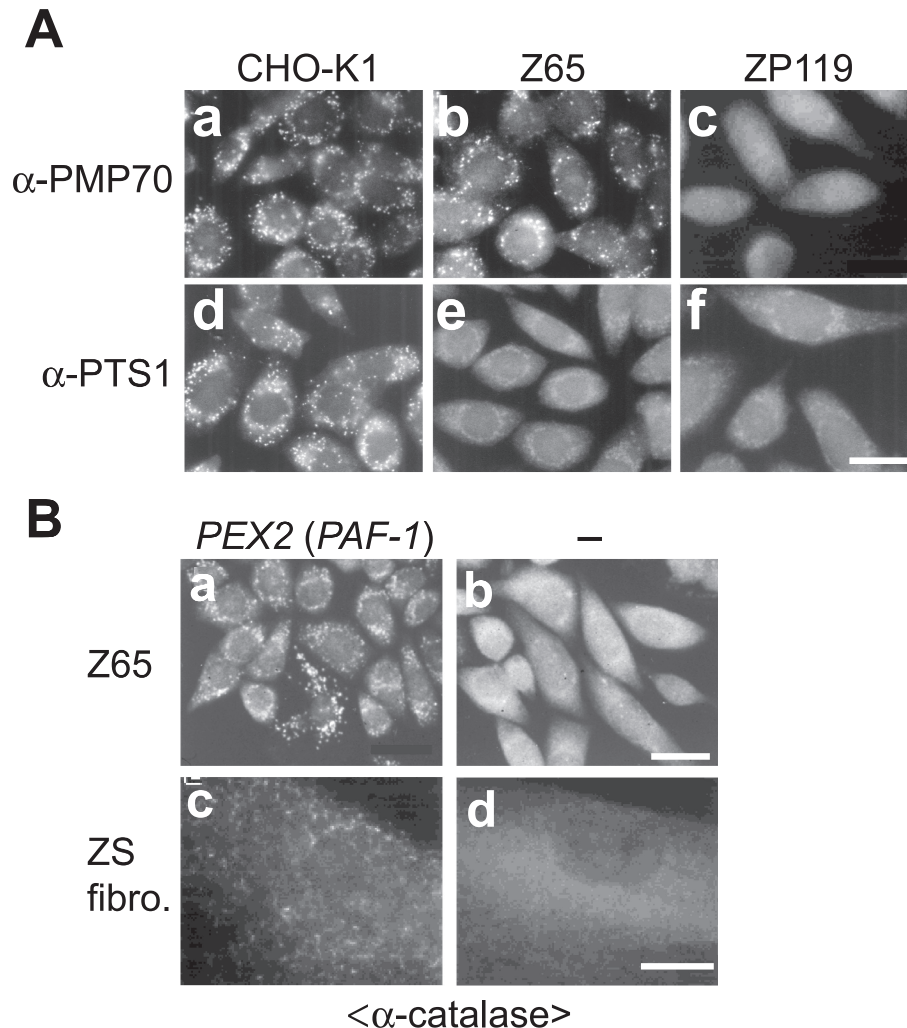

Fig. 1. Morphology of peroxisomes in CHO cell mutants defective in peroxisome biogenesis and pathogenic gene cloning of PBDs. (A) CHO cells are stained with antibodies to PMP70 (a-c) and PTS1 (d-f). Cells are as indicated at the top. Scale bar, 20 µm. *pex2* Z65 contains PMP70-positive peroxisomal remnants, whereas *pex19* ZP119 is absent from such peroxisome ghosts, indicative of the defect of membrane protein import. PTS1 proteins are discernible in the cytosol in *pex2* Z65 and *pex19* ZP119 cells, in contrast to the wild-type CHO-K1 cells where PTS1 proteins are in peroxisomes. (B) Cloning of pathogenic gene responsible for PBD. Peroxisome-restoring *PEX* genes were isolated by functional phenotype-complementation assay using CHO mutants. Restoration of peroxisomes was searched by transfection of rat liver cDNA library (a) in Z65 (b). Transformed cells positive in catalase import contained *PEX2* (formerly *PAF-1*). In fibroblasts from a patient with ZS of CG10 (d), expression of *PEX2* restored the impaired import of catalase (c). Scale bar, 20 µm (a and b); 30 µm (c and d).

two membrane-spanning segments and a RING finger motif, C_3HC_4,[37] termed peroxisome assembly factor-1 (PAF-1)[35] (Table 3; Fig. 1B). *PAF-1* was unified as *PEX2* in 1996.[33] Expression of *PEX2* (called *Zellweger gene*) in fibroblasts from a ZS patient of CG10 (F) complemented the impaired peroxisome biogenesis[38] (Fig. 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.[38]

A more practical approach, *i.e.*, a transient expression assay skipping the revertant selection by P12/UV,[39] was also developed for further isolation of *PEX* cDNAs including nine others, *PEX1*, *PEX3*, *PEX5*, *PEX6*, *PEX12*, *PEX13*, *PEX14*, *PEX19*, and *PEX26*[21),34),40)–48] (Table 3; Fig. 2). Human *PEX5*,[49),50] *PEX14*,[51] and *PEX19* (*PXF*)[52] were earlier identified. These *PEX*s were shown to be the pathogenic genes involved in PBDs of nine CGs[22),24),34),53),54] (Table 3).

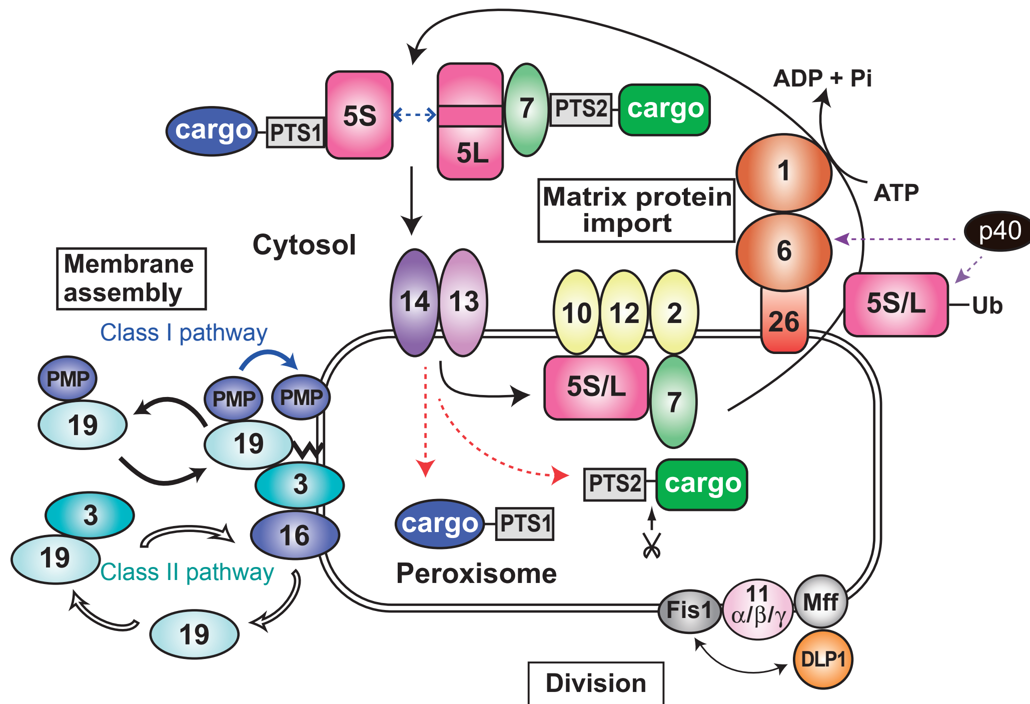

Fig. 2. A schematic view of peroxisome biogenesis in mammalian cells. The subcellular localization and molecular characteristics of peroxins are shown. Peroxins are classified into three groups: 1) peroxins including Pex3p, Pex16p and Pex19p that are responsible for peroxisome membrane assembly via classes I and II pathways required for matrix protein import; 2) those required for matrix protein import; 3) those such as three forms of Pex11p, Pex11pα, Pex11pβ, and Pex11pγ, apparently involved in peroxisome division where DLP1, Mff, and Fis1 coordinately function. PTS1 and PTS2 matrix proteins are recognized by Pex5p and Pex7p, respectively, in the cytoplasm. Two isoforms, Pex5pS and Pex5pL with an internal 37-amino-acid insertion, 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 constituents, named translocation complex consisting of the RING peroxins, Pex2p, Pex10p, and Pex12p. Both Pex5p and Pex7p finally shuttle back to the cytosol. At the terminal step of the matrix protein import reaction, Pex1p and Pex6p of the AAA family catalyze the export of Pex5p, where Cys-monoubiquitination of Pex5p is a prerequisite to the Pex5p exit. Moreover, a cytosolic factor, AWP1/ZFAND6 (p40), is involved in the export of Ub-Pex5p in mammals.

2.2.2. Expressed sequence tag homology search. An alternative strategy, *i.e.*, the homology search by screening the expressed sequence tag database using yeast *PEX* genes, successfully made it feasible to isolate human orthologue genes responsible for PBDs:[22),24),34)] *PEX1*,[55),56)] *PEX3*,[57)] *PEX5*,[58)] *PEX6*,[59)] *PEX7*,[60)–62)] *PEX10*,[63),64)] *PEX12*,[65)] *PEX13*,[66)] *PEX14*,[67)] and *PEX16*.[68),69)]

A PBD patient of the 14th CG, CG16, was recently identified with pathogenic gene *PEX11β*.[32)] Therefore, 14 *PEX*s are now shown to be responsible for PBDs of 14 distinct CGs[5),22),24),27),53),70)] (Table 3).

2.2.3. Genotypes of RCDP. Several recent findings classified RCDP into five genotypes, of which responsible pathogenic genes are delineated. RCDP type 1 is caused by mutation of *PEX7* encoding the peroxisome-targeting signal 2 (PTS2) receptor;[60)–62)] types 2, 3, and 4 are impaired in three genes, *DHAPAT*, *ADAPS*, and *FAR1*, encoding peroxisomal enzymes, DHAP-ATase, alkyl-DHAP synthase, and fatty acyl-CoA reductase, respectively, involved in the synthesis of plasmalogens;[71)–73)] type 5 manifesting a mutation of Pex5pL[74)] that transports PTS1 proteins and Pex7p-PTS2 protein complex to peroxisomes.[75)]

2.2.4. Genotype–Phenotype Relationships. Patients with milder form of PBDs, NALD and IRD, tend to manifest less severe biochemical abnormalities, whose specimen including skin fibroblasts likely contain residual peroxisomes, occasionally termed

mosaicism. However, clinical severity or prognosis of patients with PBDs cannot be readily predicted only on the basis of biochemical analyses. Various types of mutations such as nonsense point mutations, missense mutations, insertion and deletion of nucleotides mostly with concomitant frameshifts, splicing defects, etc. in both homozygotic and heterozygotic alleles have been identified in PBD patients. Patients with severe ZS tend to carry severe mutation such as nonsense mutations, frameshifts, and deletions, while many patients with NALD or IRD patients frequently harbor missense mutations.[76),77)] There is also a relationship between severe phenotype and absence of peroxisomal ghosts. Defects of *PEX3*, *PEX16* and *PEX19* encoding membrane-assembly peroxins lead to absence of ghosts and cause ZS phenotypes.[22),24),34)] Many cell lines from milder PBD patients, those with NALD and IRD with missense *PEX* mutations, showed a temperature-sensitive (*ts*) phenotype, restoration of peroxisome biogenesis at 30 °C[78)–81)] (Table 3).

Search for pathogenic genes responsible for all PBD CGs is accomplished.[82)] Prenatal DNA diagnosis using *PEX* genes is now possible for PBDs of all 14 CGs.

3. Biogenesis of peroxisomes

3.1. Membrane biogenesis.

3.1.1. Peroxins essential for membrane assembly of peroxisomes. Three mammalian peroxins, Pex3p, Pex16p, and Pex19p, exclusively required for peroxisomal membrane assembly were isolated by the functional phenotype-complementation assay on *pex3* and *pex19* CHO cell mutants[41),47)] and the EST database search using yeast *PEX* genes.[52),57),68),69)] Malfunctions of Pex3p, Pex16p, and Pex19p, causes the most severe PBD, ZS, of three CGs, CG12 (G), CG9 (D), and CG14 (J), respectively[22),24),34),83)] (Table 3).

Pex3p, Pex16p, and Pex19p were identified as essential factors for assembly of peroxisomal integral membrane proteins (PMPs) in several species including humans[25),47),68),69),84)–89)] (Fig. 2). They function as essential factors in the transport process of membrane proteins and membrane vesicle assembly in a concerted manner. Pex19p is 33-kDa farnesylated protein harboring farnesylation CAAX box motif and localized mostly in the cytosol and only partly anchored to peroxisomal membranes.[47)] 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.[90),91)] Pex19p forms stable Pex19p-PMP complexes except for Pex3p in the cytosol with a broad PMP-binding specificity.[91)–93)] Pex3p, 42-kDa integral membrane protein of peroxisomes, serves as the membrane-anchoring site for Pex19p-PMP complexes, termed Class I pathway.[91),94)] Very recently, we demonstrated that translocation of PMPs including topologically distinct PMPs such as multi-membrane spanning PMPs and an N-terminally signal-anchored protein via the class I pathway is a common event in mammalian cells.[91)]

Pex16p, a protein absent in most yeasts,[69),95)] functions as the receptor for Pex19p complexes with newly synthesized Pex3p,[94)] named Class II pathway (Fig. 2). The function of Pex16p is not conserved between different species. It is noteworthy that C-tailed anchor-type peroxin Pex26p, the recruiter of Pex1p-Pex6p complex, is transported in a class I pathway,[96)] which is distinct from the GET3-dependent topogenesis of yeast Pex15p, a functional orthologue of Pex26p.[97)]

At the step of docking of a cytosolic Pex19p-PMP complex onto Pex3p, Pex19p unloads the cargo PMP and shuttles back to the cytosol for a next round of PMP transport, while the released PMP integrates into the membrane. The membrane insertion of PMPs proceeds in the absence of ATP.[96),98)–100)] Pex19p and Pex3p apparently facilitate the insertion of transmembrane domains in a concerted manner.[101),102)] Investigation of molecular mechanisms underlying the membrane integration of the cargo PMPs is under way.

3.1.2. ER is involved in peroxisome biogenesis? In regard to peroxisomal membrane assembly, the concepts of the Pex19p- and Pex16p-dependent direct import as well as the ER-dependent indirect import have recently emerged.[94),103)] ER was postulated to provide the initial 'seed' for recruiting Pex3p and Pex16p required for peroxisome assembly.[104)–106)] Several groups suggested a different view of peroxisomal membrane biogenesis that peroxisomes are formed from ER[107),108)] upon induction of Pex3p;[104),109),110)] another study[111)] proposed that all peroxisomal membrane proteins are transported via ER. Several peroxisomal membrane proteins might be transported to peroxisomes via ER,[112)–114)] implying a semi-autonomous property of peroxisomes. A recent proximity-specific ribosome profiling suggested that many PMPs are translated at the ER in both mammalian and yeast cells, implying that they are plausible substrates for the indirect route.[115)] Interestingly, several PMPs seem to target to peroxisomes

both directly from the cytosol[91),94),96),116)] and indirectly via the ER.[103),117),118)]

However, the significance of such observations remains under debate. A study[119)] suggested 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. 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.[94)] Moreover, we very recently provided several lines of evidence that most, if not all, mammalian PMPs are indeed authentic substrates for the Pex19p- and Pex3p-mediated class I direct pathway.[91)] At any event, future investigations on whether the two distinct routes exist simultaneously in cells and when cells use these routes are required for comprehensive understanding of PMP biogenesis.[24),83),105),106)]

3.2. 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[24),34),90)] (Fig. 2).

3.2.1. PTS import receptors. PTS1 and PTS2 proteins are recognized by Pex5p and Pex7p, respectively, in the cytoplasm. Two isoforms of Pex5p, Pex5pS and Pex5pL with an internal 37-amino-acid insertion, are identified in mammals. PTS1 proteins are transported by homo- and hetero-oligomers of Pex5pS and Pex5pL to peroxisomes, where Pex14p of an 800-kDa complex functions as the initial Pex5p-docking site (Fig. 2). Pex5pL translocates the Pex7p-PTS2 protein complex to Pex14p.[120),121)] After releasing the cargoes, Pex5p and Pex7p translocate to a 500-kDa 'translocation complex' consisting of the RING peroxins, Pex2p, Pex10p, and Pex12p.[121)] Both Pex5p and Pex7p finally translocate back to the cytosol.[121)–126)] 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.[121),124),127)]

3.2.2. Peroxisome-cytoplasmic shuttling of import receptors. Mono-ubiquitination via the thioester bond of the conserved cysteine residue at position 11 in the N-terminal region of Pex5p (Ub-Pex5p) is a prerequisite for the Pex5p recycling, *i.e.*, in the export step from peroxisomes to the cytosol,[128)–131)] as in yeast[132),133)] (Fig. 2). Moreover, a cytosolic factor, AWP1/ZFAND6 involved in the export of Ub-Pex5p is identified in mammals;[131)] USP9X and Ubp15 are suggested as a potential deubiquitinase in mammals[134)] and yeast,[135)] respectively. A distinct redox state may affect the recycling of Pex5p requiring Cys-ubiquitination, thereby being as a possible cause to the phenotype of deficiency in matrix protein import in *PEX*-defective cells.[136)]

4. Gene defects of proteins for peroxisomal morphogenesis

Three isoforms of Pex11p family, Pex11pα,[137),138)] Pex11pβ,[139)–142)] and Pex11pγ,[138),143)] are identified as factors involved in morphogenesis of peroxisomes in mammals.[142),144)–147)] In mammalian cells, dynamin-like protein 1 (DLP1),[148)–151)] Fis1,[144),152)] and mitochondrial fission factor (Mff)[147),153)–155)] are shown to be involved in the fission of peroxisomes[156)] (Fig. 2).

We first reported a CHO cell mutant ZP121 in mammalian cells that was impaired in DLP1 with one point dominant-negative mutation at G363D in the middle region.[150)] With respect to peroxisomal dysmorphogenesis in humans, only three patients have been identified with a different defect in two proteins involved in the proliferation and division of peroxisomes. The first reported patient was a severely affected female patient, who died one month after the birth and postmortally was found to have a dominant-negative heterozygous mutation at G395D in DLP1 resulted in a severe fission defect of both peroxisomes and mitochondria.[151)] The second patient with dysfunctional Dnm1L (DLP1) harboring G362D mutation was most recently reported.[157)] The first patient with a defect of peroxisomal division due to a homozygous non-sense mutation in the *PEX11β* gene was recently reported as the 14th CG (CG16) of PBDs[32)] (Table 3).

5. Turnover of peroxisomes

Several hundred peroxisomes in mammalian cells are maintained by peroxisome homeostasis, a balance between the biogenesis and turnover of peroxisomes. A form of autophagy specific for peroxisomes, named pexophagy, is the main pathway for peroxisome degradation in mammals.[158)] Pexophagy is well studied at a molecular level in yeast because the strong peroxisome-induction condition and the sensitive detection and gene screening systems of pexophagy are established.[159)–161)] In contrast to the yeast system, however, molecular mechanisms of mammalian pexophagy remained largely unknown for long time.

In recent years, several reports described six different types of inducing conditions for pexophagy in mammalian cells by different stimuli, including (a) nutrient-replenishment for short period of starvation,[162),163)] (b) Ub-anchored peroxisomal membrane proteins,[164)] (c) NBR1, one of the autophagy adaptor proteins,[165)] (d) Pex3p,[166)] (e) mono-Ub-Pex5p,[167)] and (f) H_2O_2.[168)] Common aspects include that peroxisomal ubiquitination is recognized by autophagy adaptor proteins, p62 and/or NBR1, and that peroxisomes are then connected to autophagy machineries. Such useful and effective systems will shed light to mechanisms of mammalian pexophagy.

6. Perspective

Mammalian cell mutants of 15 CGs defective of peroxisome biogenesis have been identified, including PBD patients' fibroblasts and CHO mutant cell lines (Table 3). Pathogenic genes are now elucidated for all 14 CGs of PBDs. 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 reported. Investigations using the cloned peroxins and *pex* mutants including CHO cell mutants, cell lines from PBD patients, and *PEX* gene-knockout mice[141),169)–172)] will shed light on the mechanisms involved in biogenesis, morphogenesis, and homeostasis of peroxisomes and pathogenesis of PBDs.

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Profile

Yukio Fujiki was born in Fukuoka Prefecture in 1948 and graduated from Kyushu University in 1971. He received Ph.D. degree in 1976 and worked as a post-doctoral fellow in Cornell University Medical College and then as a research associate at C. de Duve's lab in the Rockefeller University, where he became an Assistant Professor in 1980. He came back to Japan in 1985 to work for Meiji Institute of Health Science and served as a Head and Chief Scientist at Department of Molecular Cell Biology. He became a Professor at Faculty of Science, Kyushu University in 1994 and then was appointed Distinguished Professor in 2009 and Professor Emeritus in 2013. He also served as Executive Vice President of Kyushu University from 2010 to 2014, and as Administrative Director of International Institute for Carbon-Neutral Energy Research, Kyushu University from 2013 to 2014. He continues his research at Medical Institute of Bioregulation as Professor from 2014.

He established one-step membrane isolation method called sodium carbonate or alkaline extraction method. This method has been widely used for cell membrane isolation and for assessing the integration of proteins into membrane. He has been working on intracellular organelle homeostasis by taking peroxisome as a model system. The functional consequence of human peroxisomes is highlighted by fatal genetic peroxisome biogenesis disorders (PBD), including Zellweger (cerebro-hepato-renal) syndrome, all of which are linked to a failure of peroxisome assembly. Peroxisome assembly in mammals including humans requires more than 14 *PEX* gene products termed peroxins. Fujiki and his colleagues isolated 11 *PEX* genes responsible for PBD. Fujiki's group tackles the problems involving membrane assembly, matrix protein import, morphogenesis, and homeostasis of peroxisomes, which are tightly linked to cell functions.