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REVIEW

Recent insights into peroxisome biogenesis and associated diseases

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ABSTRACT

Peroxisomes are single-membrane organelles present in eukaryotes. The functional importance of peroxisomes in humans is represented by peroxisome-deficient peroxisome biogenesis disorders (PBDs), including Zellweger syndrome. Defects in the genes that encode the 14 peroxins that are required for peroxisomal membrane assembly, matrix protein import and division have been identified in PBDs. A number of recent findings have advanced our understanding of the biology, physiology and consequences of functional defects in peroxisomes. In this Review, we discuss a cooperative cell defense mechanisms against oxidative stress that involves the localization of BAK (also known as BAK1) to peroxisomes, which alters peroxisomal membrane permeability, resulting in the export of catalase, a peroxisomal enzyme. Another important recent finding is the discovery of a nucleoside diphosphate kinase-like protein that has been shown to be essential for how the energy GTP is generated and provided for the fission of peroxisomes. With regard to PBDs, we newly identified a mild mutation, Pex26-F51L that causes only hearing loss. We will also discuss findings from a new PBD model mouse defective in Pex14, which manifested dysregulation of the BDNF–TrkB pathway, an essential signaling pathway in cerebellar morphogenesis. Here, we thus aim to provide a current view of peroxisome biogenesis and the molecular pathogenesis of PBDs.

KEY WORDS: Peroxisome, Oxidative stress, VDAC2, BAK, Nucleoside diphosphate kinase family, Peroxisome biogenesis disorder, BDNF, TrkB

Introduction

The peroxisome is a ubiquitous, single-membrane-bounded intracellular organelle present in almost all, if not all, eukaryotes. It is classically defined as a subcellular organelle containing catalase and at least one hydrogen peroxide (H_2O_2)-producing oxidase (de Duve and Baudhuin, 1966). Peroxisome functions in the catabolism of a wide variety of substrates such as fatty acids, D-amino acids, L-α-hydroxy acids, uric acid and polyamine. Several human genetic disorders linked to an apparent absence of peroxisomes appear to be related to various biochemical dysfunctions. It is also known that the peroxisome plays crucial metabolic roles, including the catabolism of very long chain fatty acids through the β-oxidation system, biosynthesis of ether-linked glycerolipids, such as plasmalogens, metabolism of cholesterol and phytanic acid, and synthesis of bile acids (Heymans et al., 1983; Moser, 1987). Among these disorders related to peroxisome dysfunction, cerebro-hepato-renal syndrome (Zellweger syndrome) is a typical, severe disease (Goidfischer and Reddy, 1984; Zellweger et al., 1988). Many lines of biochemical and morphological evidence are consistent with the idea that peroxisomes are formed by division of pre-existing peroxisomes after post-translational import of newly synthesized proteins (see Lazarow and Fujiki, 1985; Borst, 1986 for reviews). These oxidative metabolic pathways produce H_2O_2, which is subsequently decomposed *in situ* by peroxisomal catalase. The importance of peroxisomal function is highlighted by fatal human genetic peroxisomal biogenesis disorders (PBDs), including Zellweger spectrum disorders (ZSDs), which account for ∼80% of the patients with PBDs, including Heimler syndrome (Ratbi et al., 2015) and rhizomelic chondrodysplasia punctate (RCDP) (Weller et al., 2003). PBD patients manifest progressive metabolic disease, as well as developmental abnormalities that produce distinct dysmorphic features, including abnormal morphology of the central nervous system (CNS) (Berger et al., 2016). ZSDs comprise the most severe Zellweger syndrome (or cerebro-hepato-renal syndrome) (Goldfischer et al., 1973), the less severe neonatal adrenoleukodystrophy (NALD), and milder infantile Refsum disease (IRD) (Weller et al., 2003). Mutations in five different genes have been linked to RCDPs (Waterham et al., 2016; Honsho and Fujiki, 2017), including RCDP type 1 (RCDP1) with a deficiency in peroxisomal targeting signal 2 (PTS2)-transporter Pex7 (see below). Peroxisomal matrix proteins harbor either a peroxisomal targeting signal 1 (PTS1), which is a C-terminal SKL motif, or an N-terminal PTS2, which is the degenerate signal -R/KX5Q/HL, and are synthesized on free ribosomes in the cytosol and post-translationally transported to peroxisomes by the cytosolic receptors Pex5 or Pex7 (Fujiki, 2016; Platta et al., 2016) (Fig. 1). Inherited mutations in the genes encoding the peroxisomal enzymes essential for the synthesis of plasmalogens, including dihydroxyacetonephosphate acyltransferase, alkylglycerone phosphate synthase and fatty acyl-CoA reductase 1, cause RCDP types 2, 3, and 4, respectively, whereas a dysfunction of Pex7 and Pex5L, the longer form of Pex5, causes RCDP types 1 and 5, respectively (Waterham et al., 2016; Honsho and Fujiki, 2017).

Genetic heterogeneity comprising 14 complementation groups is found for PBDs, including ZSDs and RCDP1 (Matsumoto et al., 2003a; Thoms and Gaertner, 2012; Ebberink et al., 2012). To date, all of 14 genes responsible for these PBDs, called peroxin (*PEX*) genes, have been identified (Matsumoto et al., 2003a; Thoms and

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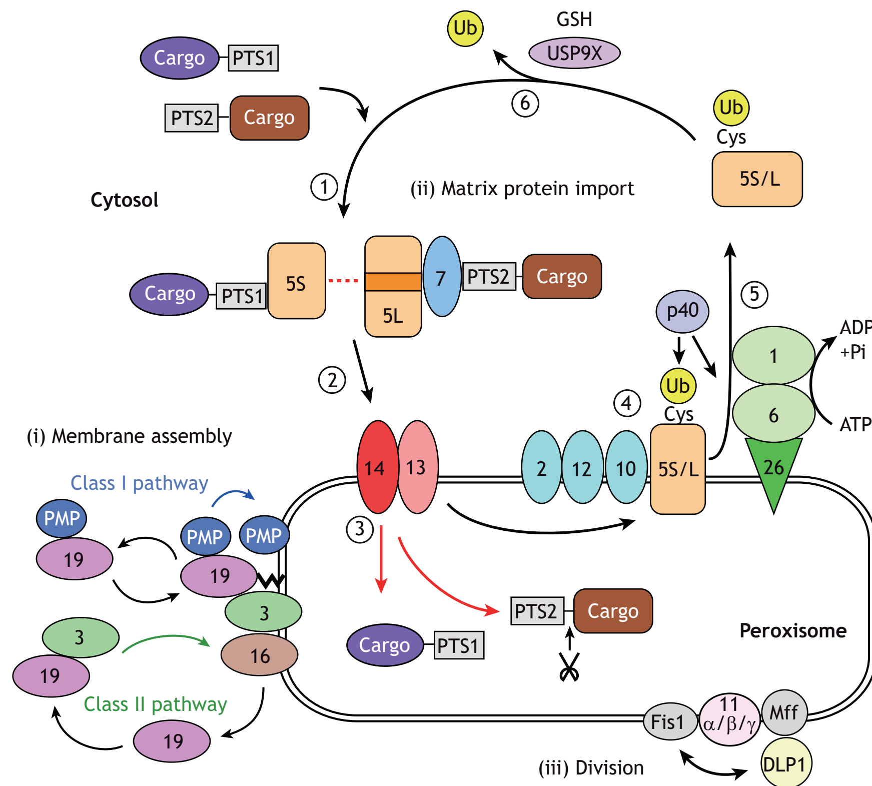

Fig. 1. A schematic view of peroxisome biogenesis in mammalian cells. Overview of the subcellular localization and molecular characteristics of peroxins. Peroxins are classified into three groups: (i) those, including Pex3, Pex16 and Pex19, which are responsible for peroxisome membrane assembly through the class I and class II pathways; (ii) those required for matrix protein import; and (iii) the different isoforms of Pex11, such as Pex11α, Pex11β, and Pex11γ, that are involved in peroxisome division, together with DLP1, Mff and Fis1. (i) In membrane protein assembly, the cytoplasmic protein Pex19 binds and transports peroxisomal membrane proteins to the membrane-anchoring site Pex3 (class I pathway), and Pex16 functions as the receptor for Pex19 complexes with newly synthesized Pex3 (class II pathway). (ii) For matrix protein import, PTS1- and PTS2-containing matrix proteins are recognized in the cytoplasm by Pex5 and Pex7, respectively (step 1). PTS1 proteins are transported to peroxisomes by homo- and hetero-oligomers of Pex5S and Pex5L; there, Pex14 functions as a convergent, initial docking site of the 'protein import machinery' translocon (step 2). The Pex5L–Pex7–PTS2 complex is then recruited to Pex14 (in complex with Pex13) in the peroxisomal membrane, and the PTS1 and PTS2 cargo proteins are released either at the inner surface and/or the inside of peroxisomes (step 3), downstream of Pex14 and upstream of Pex13. Pex5 and Pex7 subsequently interact with the RING-containing peroxins, Pex2, Pex10 and Pex12, in the membrane before they finally shuttle back to the cytosol (step 4). In the terminal step of matrix protein import, a complex between Pex1 and Pex6, which both have AAA ATPase activity, interact with Pex26 and catalyzes the export of Pex5; here, Cys-monoubiquitylation of Pex5 (Ub-Pex5) is a prerequisite for the exit of Pex5 from peroxisomes (step 5). In addition, in mammals, the cytosolic factor AWP1/ZFAND6 (p40) is involved in the export of Ub-Pex5. Exported Ub-Pex5 in the cytosol is deubiquitylated by the deubiquitylating enzyme USP9X and/or glutathione (GSH) for a next round of matrix protein import (step 6). (iii) Three forms of Pex11, Pex11α, Pex11β and Pex11γ, are involved in peroxisome proliferation where DLP1, Mff and Fis1 coordinately function (see text). The figure is adapted and modified from Fujiki (2016).

Gaertner, 2012; Ebberink et al., 2012; Fujiki et al., 2014b; Fujiki, 2016; Waterham et al., 2016) (Table 1). Peroxins are classified into three groups: (1) Pex3, Pex16 and Pex19, which are essential for peroxisome membrane assembly via classes I and II pathways; (2) ten peroxins that are required for matrix protein import; and (3) the different isoforms of Pex11, namely Pex11α, Pex11β and Pex11γ, which are involved in peroxisome division together with dynamin-like protein 1 (DLP1, also known as DNM1L), mitochondrial fission factor (Mff) and mitochondrial fission protein 1 (Fis1) (Fujiki, 2016; Platta et al., 2016; Farré et al., 2019) (Fig. 1). However, despite the identification of the peroxins, several aspects of peroxisome function remain incompletely understood, such as regulation of peroxisome number in cells and mechanistic insight to pathogenesis of ZSDs.

In this Review, we will address three important issues with regard to peroxisome function, including how peroxisomes counteract cellular oxidative stresses, how the GTP that is required for the division machineries of peroxisomes and mitochondria is generated, and what are the pathogenic mechanisms underlying PBDs.

Peroxisomes counteract oxidative stress – cell death and survival against oxidative stress

Although reactive oxygen species (ROS) are formed as a natural by-product of normal metabolism of oxygen, oxidative stress occurs when the generation of ROS exceeds the capacity of antioxidant defense. Mitochondria are widely recognized as a source of ROS in animal cells, but peroxisomes are another source of ROS, as H_2O_2 can be generated by peroxisomal oxidation, such as fatty-acid β-oxidation, and decomposed by catalase. We discuss below our serendipitous finding that the proapoptotic factor BAK (also known as BAK1) regulates subcellular localization of catalase, implying a strategy against oxidative cellular stresses.

BAK mediates peroxisomal catalase export

In the 1990s, we isolated a peroxisome-deficient Chinese hamster ovary (CHO) cell mutant, ZP114, which was impaired in matrix protein import (Tateishi et al., 1997), but the underlying mechanism was unclear. Using a functional forward genetic screening strategy, we recently identified voltage-dependent anion-selective channel

Table 1. Mammalian *PEX* genes involved in peroxisome biogenesis and responsible for peroxisome deficiency disorders

	CG[a]					Peroxin	
Gene	US/EU	Japan	PBD	CHO mutants	Ps-memb. biogenesis[b]	(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[c]	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

*Temperature-sensitive phenotype. [a]Complementation group; [b]peroxisomal membrane assembly is normal (+) or impaired (–); [c]besides *PEX11β*, genes including *DLP1*, *Mff*, *Fis1* and *GDAP1* are also involved in fission process of peroxisome (see text). DP, docking protein; PMP, peroxisome membrane protein; TPR, tetratricopeptide repeat. For a review on the cloning of *PEX* genes, see Waterham and Ebberink (2012).

protein 2 (VDAC2) as being responsible for the defect in peroxisome biogenesis in the mutant cell ZP114 (Hosoi et al., 2017), in which the pro-apoptotic Bcl-2 protein BAK accumulates to remnants of peroxisomal membranes. We then attempted to delineate the role of BAK in peroxisome biogenesis. Knockdown (KD) of *BAK* or overexpression of the BAK inhibitors BCL-X_L (the large isoform encoded by *BCL2L1*) and MCL-1 restored the impaired peroxisome biogenesis in ZP114 cells, indicating that in the absence of VDAC2, BAK is sequestered from mitochondria to peroxisomes, where it induces their permeabilization, thereby giving rise to peroxisome deficiency (Hosoi et al., 2017). In normal cells, including CHO-K1 cells under normal conditions, catalase is mainly localized in peroxisomes, but some is also present in the cytosol (Baudhuin et al., 1964; Hosoi et al., 2017). Surprisingly, in *BAK*-KD wild-type CHO-K1 cells, the amount of cytosolic catalase was significantly and specifically reduced, suggesting that BAK is involved in cytosolic localization of catalase. Conversely, activation of BAK in CHO-K1 cells by overexpression of either one of the pro-apoptotic BH3-only proteins PUMA or BIM (also known as BBC3 and BCL2L11, respectively), but not BAD, released catalase from peroxisomes (Hosoi et al., 2017). Collectively, these findings strongly suggest that BAK localizes to peroxisomes and regulates peroxisome membrane permeability and catalase export (Fujiki et al., 2017; Hosoi et al., 2017) (Fig. 2). Such a mechanism underlying the subcellular localization of catalase has been further discussed in a spotlight article (Chipuk and Luna–Vargas, 2017).

Under intolerable cellular stress, such as oxidative stress, cells commit to apoptosis by compromising the integrity of the mitochondrial outer membrane, so-called mitochondrial outer membrane permeabilization (MOMP) (Wang, 2001; Newmeyer and Ferguson-Miller, 2003; Tait and Green, 2010). MOMP leads to the release of intermembrane-space proteins, including cytochrome *c*, followed by activation of caspases, which are responsible for the progression of apoptosis (Wang, 2001; Newmeyer and Ferguson-Miller, 2003; Tait and Green, 2010). The BCL-2 family proteins regulate apoptosis by controlling MOMP (Czabotar et al., 2014). Within the mitochondrial pathway of apoptosis, VDAC2 controls both the localization and proapoptotic activity of BAK. A potentially prosurvival mechanism that is characterized by permeabilization of the peroxisome membrane to release catalase thus contrasts with commitment of the cell to the mitochondrial pathway of apoptosis by promoting cytochrome *c* release. Thus BAK-dependent membrane permeabilization is shared between mitochondria and peroxisomes, but with different outcomes. The

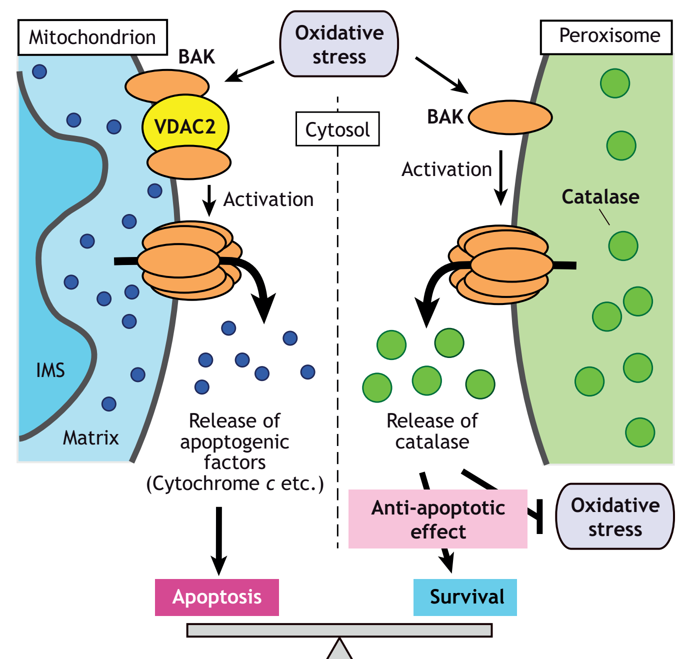

Fig. 2. BAK-dependent control of catalase release from peroxisomes. BAK is mostly localized at the mitochondrial outer membrane (MOM) where it is kept in an inactivated state by forming a complex with VDAC2 (left). Once BAK is activated upon apoptotic stimuli, it induces MOM permeabilization (MOMP) to release apoptogenic factors such as cytochrome *c*, which commit cells to undergoing apoptosis. A portion of the cellular pool of BAK is localized to peroxisomes in normal cells, where it controls the permeability of the peroxisomal membrane, leading to release of catalase to the cytosol (right). BAK-dependent membrane permeabilization is thus a mechanism that is shared between mitochondria and peroxisomes. Bona fide cytosolic catalase and the catalase released from peroxisomes through the BAK pore eliminate H_2O_2, a major causative of the oxidative stress, in the cytosol. The figure is adapted and modified from Fujiki et al. (2017) (https://www.tandfonline.com/).

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catalase that is released from peroxisomes through the BAK pore potentially eliminates H_2O_2, a major causative of the oxidative stress, in the cytosol. We therefore proposed a novel and unique regulatory mechanism of peroxisomal biogenesis mediated by the BCL-2 family protein BAK, not BAX, that entails a regulation of the localization of peroxisomal matrix proteins through alterations in peroxisomal membrane permeability in response to various cellular stress signals, rather than the modulation of protein import machinery (Hosoi et al., 2017; Fujiki et al., 2017).

Catalase is imported into the peroxisome by the PTS1 receptor Pex5, which shuttles between the cytosol and peroxisomes to import peroxisomal matrix proteins harboring the PTS1 motif (Dammai and Subramani, 2001; Miyata and Fujiki, 2005; Platta et al., 2005). Pex5 has a highly conserved cysteine at position 11, which is a redox-sensitive site of mono-ubiquitylation that is essential for export of Pex5 to the cytosol (Carvalho et al., 2007; Platta and Erdmann, 2007; Okumoto et al., 2011). In the more reductive cytosol in peroxisome-deficient mutants compared to that of normal cells (Yano et al., 2010), cysteine 11 of Pex5 is less sensitive to the redox state; it becomes more stable, is highly susceptible to oxidants, such as oxidized glutathione, and is readily inhibited by oxidative stress (Apanasets et al., 2014). More recently, cysteine 11 of Pex5 has been suggested to act as a redox sensor and reduce the import of PTS1-containing proteins, especially catalase, under oxidative stress, which leads to the retention of catalase in the cytosol (Walton et al., 2017). Thus, it is likely that the release of catalase from peroxisomes mediated by BAK (Hosoi et al., 2017) and the inhibition of its import owing to defective ubiquitylation of cysteine 11 of Pex5 under oxidative stress (Apanasets et al., 2014) cooperatively contribute to the retention of catalase in the cytosol.

Further studies regarding the physiological roles of peroxisomal BAK and cytosolic catalase could help to delineate the mechanisms underlying the regulation of the localization of peroxisomal matrix proteins that are involved in peroxisome physiology and human disease.

Morphogenesis of peroxisomes in mammals

Peroxisomes proliferate by growth and division (Lazarow and Fujiki, 1985; Thoms and Erdmann, 2005; Yan et al., 2005; Schrader and Fahimi, 2006; Fagarasanu et al., 2007; Honsho et al., 2016; Schrader et al., 2016). Peroxisomal division comprises three stages – elongation, constriction and fission – and involves Pex11β (Li and Gould, 2002), dynamin-like protein 1 (DLP1) (Li and Gould, 2003; Tanaka et al., 2006), mitochondrial fission factor (Mff) (Gandre-Babbe and van der Bliek, 2008; Itoyama et al., 2013) and mitochondrial fission 1 (Fis1) (Koch et al., 2005; Kobayashi et al., 2007), all of which are localized to peroxisomes (Fig. 1; Table 1). Except for Pex11β, these factors are shared with mitochondria (Koch and Brocard, 2012; Schrader et al., 2012; Itoyama et al., 2013). Peroxisome fission is important for its normal function. Indeed, a human disorder with impaired DLP1 function has been reported (Waterham et al., 2007). The patient, a newborn girl, manifested microcephaly, abnormal brain development, optic atrophy and hypoplasia, with a defect of the fission of both mitochondria and peroxisomes, as well as a heterozygous, dominant-negative mutation in the *DLP1* gene (Waterham et al., 2007). A poly-unsaturated fatty acid of peroxisomal β-oxidation metabolites, docosahexaenoic acid (DHA, C22:6n-3), augments hyper-oligomerization of Pex11β and induces the elongation of peroxisomes, which leads to the fission stage in control fibroblasts, suggesting that a complex mechanism strictly regulates the peroxisome abundance to a constant level under normal conditions (Itoyama et al., 2012) (Fig. 3). Translocation of DLP1, a member of the large GTPase family, from the cytosol to peroxisomes is a prerequisite for membrane fission. Mff is localized to peroxisomes, especially at the membrane-constricted regions of elongated peroxisomes (Itoyama et al., 2013). Knockdown of Mff abrogates the fission stage of peroxisomal division and leads to a failure to recruit DLP1 to peroxisomes. In contrast, ectopic expression of Mff elevates the peroxisomal targeting of DLP1 (Itoyama et al., 2013). Co-expression of Mff and Pex11β increases peroxisome abundance. Knockdown of Mff, but not Fis1, abolishes the DLP1–Pex11β interaction. Moreover, Pex11β interacts with Mff in a DLP1-dependent manner, suggesting that Mff plays a key role in the fission of the peroxisomal membrane in concert with Pex11β and DLP1 (Itoyama et al., 2013). A functional complex comprising Pex11β, Mff and DLP1 promotes Mff-mediated fission during peroxisomal division (Tanaka et al., 2006; Kobayashi et al., 2007; Delille et al., 2010; Koch et al., 2010; Itoyama et al., 2013; Yoshida et al., 2015). A patient with a homozygous non-sense mutation in the *PEX11β* (annotated by HUGO as *PEX11B*) gene has been reported, manifesting a defect of peroxisome division, but with apparently normal peroxisomal metabolism (Thoms and Gaertner, 2012; Ebberink et al., 2012). Another potential factor, ganglioside-induced differentiation-associated protein 1 (GDAP1) was suggested to be required for peroxisome fission, downstream of Pex11β and upstream of the fission steps mediated by Mff and DLP1 (Huber et al., 2013), inferring that GDAP1 mediates the interaction between Pex11β and the Mff–DLP1 complex. However, the regulatory mechanisms underlying peroxisomal division remain to be defined.

The peroxisome and mitochondrion division machinery – role of the GTP provider DYNAMO

Peroxisomes and mitochondria proliferate by fission that is mediated by a dynamin-like GTPase, DLP1 or Dnm1 (Tanaka et al., 2006; Honsho et al., 2016). During the division of peroxisomes, a part of their membrane is pinched off by constriction of ring-shaped peroxisome-dividing (POD) machinery (Imoto et al., 2017). This constriction is mediated by the dynamin-like GTPase DLP1, which requires large amounts of GTP as an energy source. However, it is unknown where GTP is supplied from and how GDP is regulated. To search for factors involved in the regulation of DLP1, we attempted to isolate and perform proteomic analysis of the division machinery of peroxisomes. To that end, we made use of a unicellular red alga, *Cyanidioschyzon merolae*, which contains only a single peroxisome, mitochondrion and plastid per cell. In *C. merolae*, division of these organelles can be highly synchronized by stimulation with cycles of light and dark (Imoto et al., 2017). Using *C. merolae*, we recently identified a 17-kDa nucleoside diphosphate (NDP) kinase-like protein, we termed dynamin-based ring motive-force organizer 1 (DYNAMO1), as a fundamental component of both the mitochondrial division (MD) and POD machineries (Imoto et al., 2018). DYNAMO1 colocalizes with DLP1 on the division machineries and mediates the generation of GTP from ATP and GDP; this enzyme activity is essential to sustain DLP1-mediated fission of the mitochondrion and peroxisome (Imoto et al., 2018).

The POD machinery has a diameter of 50 to 600 nm and is composed of dynamin-based rings and skeletal filamentous rings formed at the cytoplasmic side of peroxisomal membranes (Imoto et al., 2013). A single or double dynamin-based ring-organizing center (DOC) is formed on the POD machinery (Imoto et al., 2017) and functions as the site where dynamin-based ring formation begins (Fig. 4). DOC-mediated dynamin-based ring formation also provides the basis for dynamin-based membrane division, including

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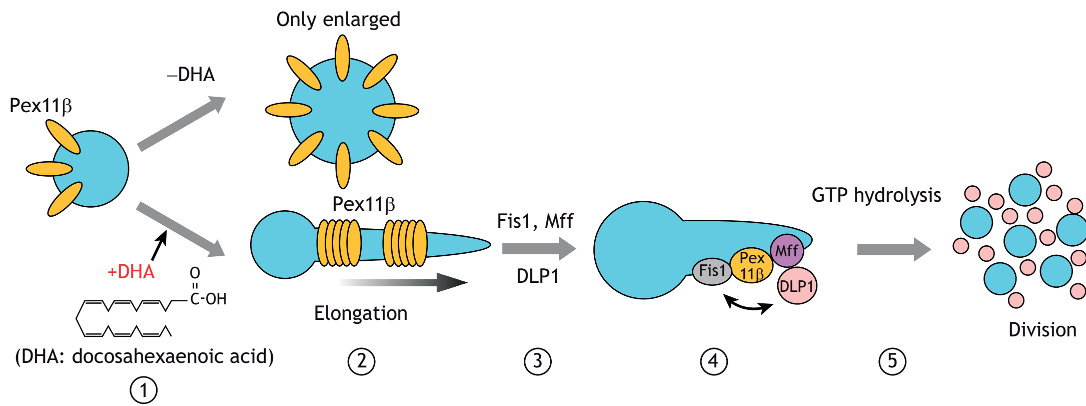

Fig. 3. Peroxisome morphogenesis in mammals. A schematic model of division of peroxisomes in mammals is represented. DHA promotes the oligomerization of Pex11β, which leads to the formation of Pex11β-rich regions in the peroxisome membrane and initiates peroxisome elongation (step 1) in one direction (step 2). Mitochondrial fission factor 1 (Mff) and mitochondrial fission protein 1 (Fis1) localize to membrane-constricted regions of elongated peroxisomes, where Mff recruits dynamin-like protein 1 (DLP1) (step 3). The resulting complex comprising constitutively expressed Pex11β, Mff and DLP1 promotes Mff-mediated fission during peroxisomal division. Pex11α and Pex11γ can be also involved in this step (step 4). The complex may in addition include Fis1, which also interacts with DLP1. The DLP1 hydrolyzes GTP, which generates GDP and provides the energy needed to cleave peroxisomal membranes, resulting in peroxisomal fission (step 5). The figure is adapted and modified with permission from Springer Nature from Fujiki et al. (2014a).

mitochondrial division (Imoto et al., 2018), plastid division (Imoto et al., 2019) and most likely vesicle endocytosis in eukaryotes. Interestingly, more recently, we identified *C. merolae* DYNAMO2, an isoform of DYNAMO1, in the cytoplasm, which contains a highly conserved NDP kinase domain and may function as a potential regulator of cellular GTP levels during the cell cycle (Imoto et al., 2019).

NDP kinases of the NME family are highly conserved in eukaryotes and are involved in many different cellular processes. Based on the primary sequence similarity, nucleoside diphosphate kinase 3 (NME3) may be a potential ortholog of DYNAMO1. An interesting recent report described a homozygous mutation in the *NME3* gene in the patient with a fatal neurodegenerative disorder (Chen et al., 2019). The authors suggest that NME3 possesses two separate functions, the stimulation of mitochondrial fusion and NDP kinase activity, implying that both functions of NME3 might be involved in causing neuronal disorder (Chen et al., 2019). It would be important to address whether NME3, which harbors a N-terminal hydrophobic segment as a putative membrane anchor, is located on peroxisomes and involved in peroxisome division. The effect of mutant NME3 on peroxisome morphogenesis will also need to be determined.

A new phenotype of ZSD – a very mild mutation in the *PEX26* gene causes severe hearing loss

Defects in *PEX* genes generally cause ZSD, which manifests in variable clinical phenotypes ranging from severe, lethal ZS to milder IRD. A number of phenotypes, including developmental delay, hypotonia, retinal dystrophy and sensorineural hearing loss, are commonly observed to varying degrees in patients with ZS, NALD and IRD (Weller et al., 2003). Defects in *PEX26* are responsible for ZSD of complementation group 8, one of the more common PBDs (Matsumoto et al., 2003a,b; Weller et al., 2005) (Table 1). By clinical exome sequencing, we recently identified an

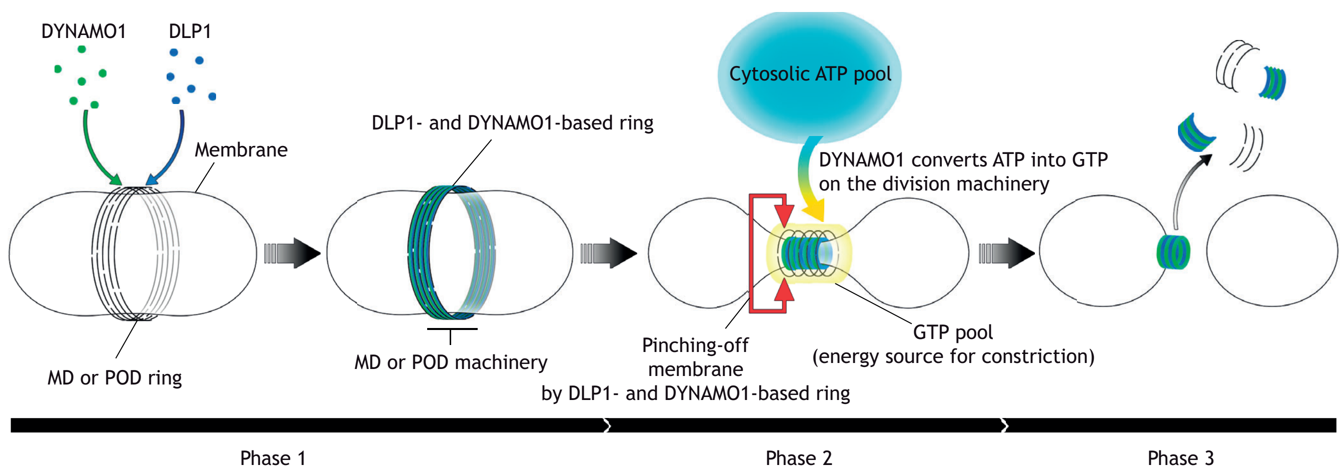

Fig. 4. Local GTP generation provides the constriction force for peroxisomal and mitochondrial division. Initially, DYNAMO1 is recruited to the division site of the mitochondrion and peroxisome, together with DLP1 (phase 1, prophase), forming ring-shaped mitochondrial division (MD) and peroxisomal division (POD) machineries, respectively. Next, DYNAMO1 converts cytosolic ATP into GTP at the ring-like structures formed by the MD and POD machineries. Upon GTP generation, the DYNAMO1–DLP1 structure generates a strong motive force to constrict and pinch off the mitochondrion or peroxisome (phase 2). After fission, the MD and POD machineries containing DYNAMO1 and DLP1 are immediately disassembled (phase 3). The figure is adopted and modified from Imoto et al. (2018) where it was published under a CC-BY-4.0 license.

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autosomal recessive missense mutation in the *PEX26* gene in a 19-year-old female of Ashkenazi Jewish pedigree with moderate to severe hearing loss (Tanaka et al., 2019). The mutation, c.153C>A (p.F51L) in *PEX26*, is homozygous in the proband and affected four siblings of seven, who share a phenotype of nonsyndromic sensorineural hearing loss with no other indications of ZSD. Pex26 is a C-tail-anchored type II membrane protein located in the peroxisomal membrane and recruits the AAA ATPase peroxins Pex1 and Pex6, which are essential for peroxisomal matrix protein import (Matsumoto et al., 2003a) (Fig. 1). The F51L mutation is located in the N-terminal region, close to the other mutations identified in patients with ZSD (Matsumoto et al., 2003a,b; Furuki et al., 2006; Waterham and Ebberink, 2012). Skin fibroblasts from this patient showed normal morphology and biogenesis of peroxisomes (Tanaka et al., 2019). However, the import rate of matrix proteins is significantly reduced and Pex26-F51L is unstable, and interacts less efficiently with Pex1–Pex6 complexes. Furthermore, in the fibroblasts of the patient, the level of DHA in plasmalogens is lowered, whereas other lipid metabolism processes, including peroxisomal fatty acid β-oxidation, are normal. Collectively, the F51L missense variant in *PEX26* causes only a mild defect in peroxisome biogenesis, which appears to lead to hearing loss (Tanaka et al., 2019). However, the molecular mechanism for how such a mild peroxisomal dysfunction with Pex26-F51L causes hearing loss remains to be defined.

It is noteworthy that potential links between peroxisomes and hearing loss have been previously reported. The *PJVK* gene encoding the gasdermin protein pejvakin (PJVK) is a causal gene of human autosomal recessive deafness (Delmaghani et al., 2015). Peroxisomal biogenesis is also known to be compromised in the cochlear cells of Persian families with hearing loss owing to a mutation *PJVK* gene, indicating that PJVK plays a role in regulating peroxisomal dynamics in cochlear cells of the inner ear (Delmaghani et al., 2015). Another study utilizing an exome sequencing approach identified biallelic mutations in *PEX1* and *PEX*6 in six families diagnosed with Heimler syndrome, a rare recessive disease with varying manifestations including sensorineural hearing loss (Ratbi et al., 2015). This study also highlights the usefulness of highly efficient exome sequencing to elucidate the pathogenesis in patients with defects in *PEX* genes, which may lead to the discovery of new functions of peroxisomes.

Mechanisms underlying pathogenesis in PBDs – dysregulation of BDNF–TrkB signaling

PBDs manifest as neurological deficits in the central nervous system, including neuronal migration defects and abnormal cerebellum development. The biochemical abnormalities, including marked reduction of plasmalogens and accumulation of very long chain fatty acids (Weller et al., 2003), are thought to be relevant to the manifestation of malformations in the CNS. However, the mechanisms underlying pathogenesis remain enigmatic.

To elucidate the pathogenesis of ZSDs, mice with inactivation of *Pex2* (Faust and Hatten, 1997), *Pex5* (Baes et al., 1997) and *Pex13* (Maxwell et al., 2003) have been established. The resulting *Pex2*, *Pex5* or *Pex13* knockout (KO) mice survive *in utero*, but all die at a several hours to a few days after birth. The deletion of these individual *Pex* genes results in a complete deficiency of peroxisomal protein import, which gave rise to severe impairment of peroxisomal fatty acid oxidation and plasmalogen synthesis, as well as abnormal morphology of the CNS with neuronal migration defects (Baes et al., 1997; Faust, 2003; Maxwell et al., 2003), similar to the reported phenotypes in patients with ZSDs including Zellweger syndrome (Volpe and Adams, 1972; Evrard et al., 1978; Powers and Moser, 1998). *Pex7*-KO mice exhibit the abnormalities in plasmalogen biosynthesis, neuronal migration in cerebral cortex, and bone ossification, which are similar to those observed in the patients with RCDP1 (Brites et al., 2003). It is worth noting that *Pex11β*-KO mice exhibit numerous pathological features of Zellweger syndrome, including a developmental delay, hypotonia, neuronal migration defects and enhanced neuronal apoptosis, even though they have no apparent defect in peroxisomal protein import and only mild defects in peroxisomal metabolic function, which is significantly different from patients and mice with Zellweger syndrome (Li et al., 2002).

As a step towards uncovering the pathological mechanisms underlying ZSDs, we recently established a new PBD mouse model that is defective in Pex14, termed the *Pex14*$^{\Delta C/\Delta C}$ mouse (Abe et al., 2018). The Pex14-defective mouse manifests neuronal migration defect in cerebral cortex, malformation of cerebellum and growth

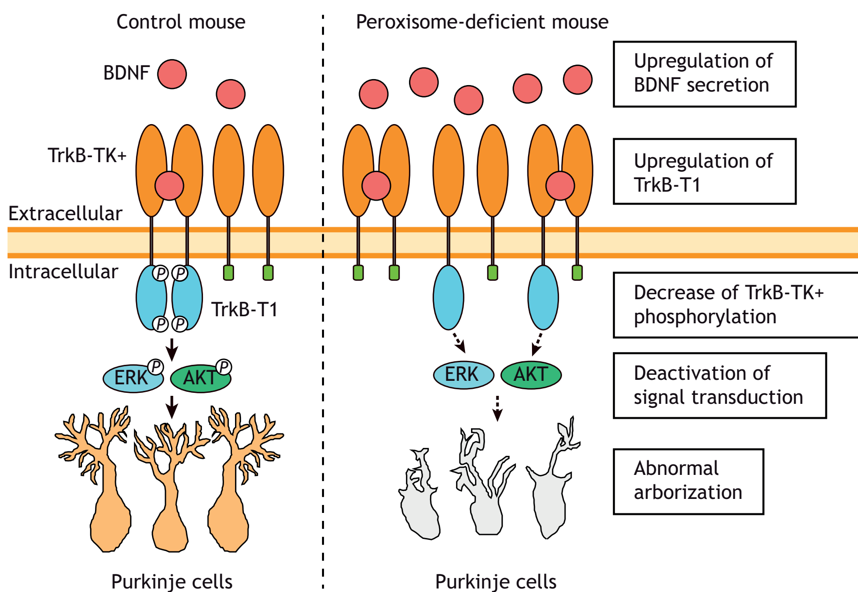

Fig. 5. A schematic model of abnormal dendritic development of Purkinje cells in the *Pex14*$^{\Delta C/\Delta C}$ mouse. In the wild-type cerebellum (left), BDNF interacts with TrkB-TK+ on the surface of Purkinje cells. The cytosolic tyrosine kinase domain (TK) then undergoes autophosphorylation and activates MAPK–ERK and PI3K–AKT signaling, leading to dendritic arborization. In the *Pex14*$^{\Delta C/\Delta C}$ mouse, BDNF levels are increased around the Purkinje cells. On the Purkinje cells of the *Pex14*$^{\Delta C/\Delta C}$ mouse (right), the inactive receptor isoform TrkB-T1 is upregulated owing to increased alternative splicing and dominant-negatively inhibits autophosphorylation of TrkB-TK+. The reduced phosphorylation of TrkB-TK+ results in inability to activate MAPK-ERK and PI3K-AKT signaling, giving rise to an abnormal dendritic development of Purkinje cells. The figure is adopted and modified from Abe et al. (2018) where it was published under a CC-BY-4.0 license.

retardations, and died shortly after birth, while peroxisome biogenesis and metabolism were only partially defective. The *Pex14*$^{\Delta C/\Delta C}$ mouse shows an impaired dendritic development of Purkinje cells in cerebellum that is caused by a dysregulation of the brain-derived neurotrophic factor (BDNF)-TrkB pathway (Abe et al., 2018) (Fig. 5). In wild-type mouse cerebellum, BDNF binds to TrkB-TK+, an active form of BDNF receptor, on the Purkinje cells and activates extracellular signal-regulated kinase (ERK) and AKT signaling. In the *Pex14*$^{\Delta C/\Delta C}$ mouse, elevated levels of BDNF together with an enhanced expression of TrkB-T1, an inactive isoform of TrkB, dominant-negatively inhibit autophosphorylation of TrkB-TK+, and thus downstream ERK and AKT signaling, thereby resulting in the malformation of Purkinje cells (Abe et al., 2018) (Fig. 5). In the cerebellum of patients with Zellweger syndrome, impairment of the Purkinje cell arborization, heterotopia of Purkinje cells in the white matter and granule cell clustering between the Purkinje cells are observed (Volpe and Adams, 1972; Evrard et al., 1978; Powers and Moser, 1998). These phenotypes resemble those observed in mice with knockout of *Pex2* (Faust and Hatten, 1997), *Pex5* (Baes et al., 1997), *Pex13* (Maxwell et al., 2003) and *Pex14* (Abe et al., 2018), including the impairment of Purkinje cell arborization and granule cell migration defects, although the heterotopic Purkinje cells are not distinct in the *Pex2*-KO mice (Faust, 2003). During cerebellar development, the BDNF–TrkB signaling pathway plays pivotal roles in Purkinje cell arborization (Minichiello and Klein, 1996; Schwartz et al., 1997; Carter et al., 2002) and in granule cell migration from the external granular layer to the internal granular layer (Zhou et al., 2007). Heterotopic Purkinje cells are also observed in the patients with milder ZSDs, NALD and IRD, which show less severe defect of peroxisomal metabolism than the patients with Zellweger syndrome (Aubourg et al., 1986; Torvik et al., 1988). Therefore, the BDNF–TrkB signaling pathway in the cerebellum is most likely susceptible to the impaired peroxisomal metabolism in ZSDs. These findings provide for the first time an insight into the mechanism underlying the cerebellar pathogenesis in PBDs (Fig. 5). Addressing how peroxisomal dysfunction affects the elevation of BDNF and TrkB-T1 in the cerebellum will shed further light on the molecular mechanisms underlying the malformation of the cerebellum in PBDs.

Perspectives

The studies discussed here clearly suggest that making use of different peroxin mutants in CHO cells and in cell lines from PBD patients, as well as *Pex* gene-KO mice can shed light on the molecular mechanisms underlying biogenesis and homeostasis of peroxisomes in organelle biology and pathogenesis of PBDs in human disease. We have highlighted here our recent insights into peroxisome biogenesis including fission, regulation of subcellular localization of catalase by BAK and the pathogenic mechanism underlying peroxisome-deficient diseases. Further studies addressing the physiological roles of peroxisomal BAK and cytosolic catalase might allow us to gain an understanding of how translocation of peroxisomal matrix proteins is regulated. Secondly, the generation and enrichment of local GTP and global GTP level are more likely regulated by DYNAMO1 and DYNAMO2, respectively, than by dynamin family members. However, it remains unclear how DYNAMO1 and DYNAMO2 regulate the supramolecular nanomachineries that influence the dynamic behavior of dynamin-regulated organelles, such as peroxisomes, mitochondria and other cellular structures. Furthermore, tackling the molecular basis of the specific phenotypes of *Pex* defects, such as how peroxisomal dysfunction mediated by knockout of *Pex14* upregulates of BDNF expression and abnormal splicing, which results in a dominant-negative variant of TrkB in Purkinje cells in cerebellum, might also lead to a better understanding of the malfunctioning of other organs, such as liver, brain and kidney in PBDs. The outcome of these studies are likely to not only contribute to a better understanding of peroxisome physiology, but also of other intracellular compartments and associated diseases that involve organelle dysregulation.

Note added in proof.

In regard to the elevation of BDNF expression in peroxisome-deficient cells, Abe et al. (Abe et al., 2020) very recently reported that cytosolic reductive states caused by a mislocalized catalase induce the elevation in BDNF secretion.

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Competing interests

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