

# The peroxisome: still a mysterious organelle

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**Abstract** More than half a century of research on peroxisomes has revealed unique features of this ubiquitous subcellular organelle, which have often been in disagreement with existing dogmas in cell biology. About 50 peroxisomal enzymes have so far been identified, which contribute to several crucial metabolic processes such as  $\beta$ -oxidation of fatty acids, biosynthesis of ether phospholipids and metabolism of reactive oxygen species, and render peroxisomes indispensable for human health and development. It became obvious that peroxisomes are highly dynamic organelles that rapidly assemble, multiply and degrade in response to metabolic needs. However, many aspects of peroxisome biology are still mysterious. This review addresses recent exciting discoveries on the biogenesis, formation and degradation of peroxisomes, on peroxisomal dynamics and division, as well as on the interaction and cross talk of peroxisomes with other subcellular compartments. Furthermore, recent advances on the role of peroxisomes in medicine and in the identification of novel peroxisomal proteins are discussed.

**Keywords** Peroxisomes · Mitochondria · Organelle dynamics · Biogenesis · Organelle cross talk · Dynamamin · Disease · Pexophagy · Fatty liver disease · Neuroinflammation

## Abbreviations

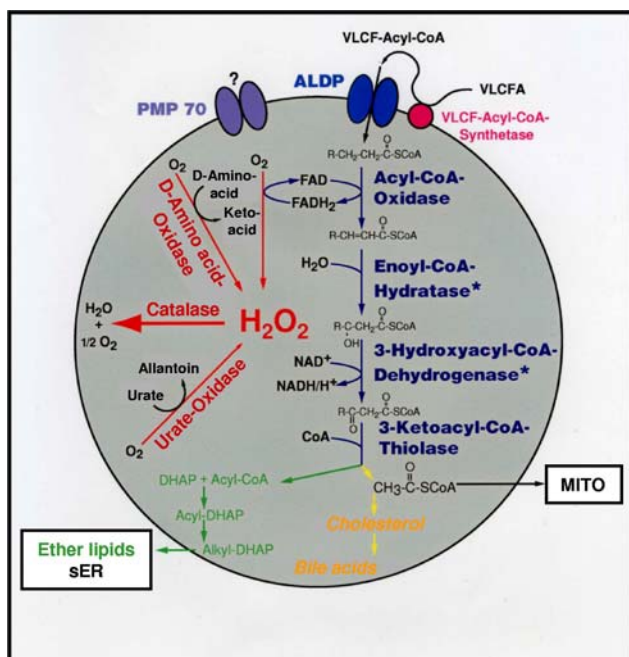
ALDP	Adrenoleukodystrophy protein
CNS	Central nervous system
ER	Endoplasmic reticulum
FLD	Fatty liver disease
PBD	Peroxisome biogenesis disorder
PEX	Peroxin
PMP	Peroxisomal membrane protein
PPAR	Peroxisome proliferator activated receptor
PTS	Peroxisomal targeting signal
ROS	Reactive oxygen species
VLCFA	Very-long-chain fatty acids

## Introduction: early milestones in peroxisome research

Peroxisomes (originally called microbodies) were discovered in 1954 using electron microscopy in mouse kidney (Rhodin 1954), 4 years before the first issue of this Journal (which in 1995 became *Histochemistry and Cell Biology*) was published. Since then the “Cinderella” among the subcellular organelles, which was once considered to be a “fossil organelle” and had been regarded as the cell’s “garbage pail”, has experienced a remarkable rise and turned into a dynamic and metabolically active cellular compartment essential for human health and development. De Duve and Baudhuin (1966) were the first who isolated peroxisomes from rat liver, and their biochemical studies led to the discovery of the colocalization of several  $H_2O_2$ -producing oxidases as well as catalase, an  $H_2O_2$ -degrading enzyme, in the matrix of peroxisomes (Fig. 1). On the basis of these findings, De Duve proposed the functional term “peroxisome”, which gradually replaced the former morphological designation, “microbody”, coined by Rhodin (Rhodin 1954). A specific cytochemical staining for peroxisomes in light and

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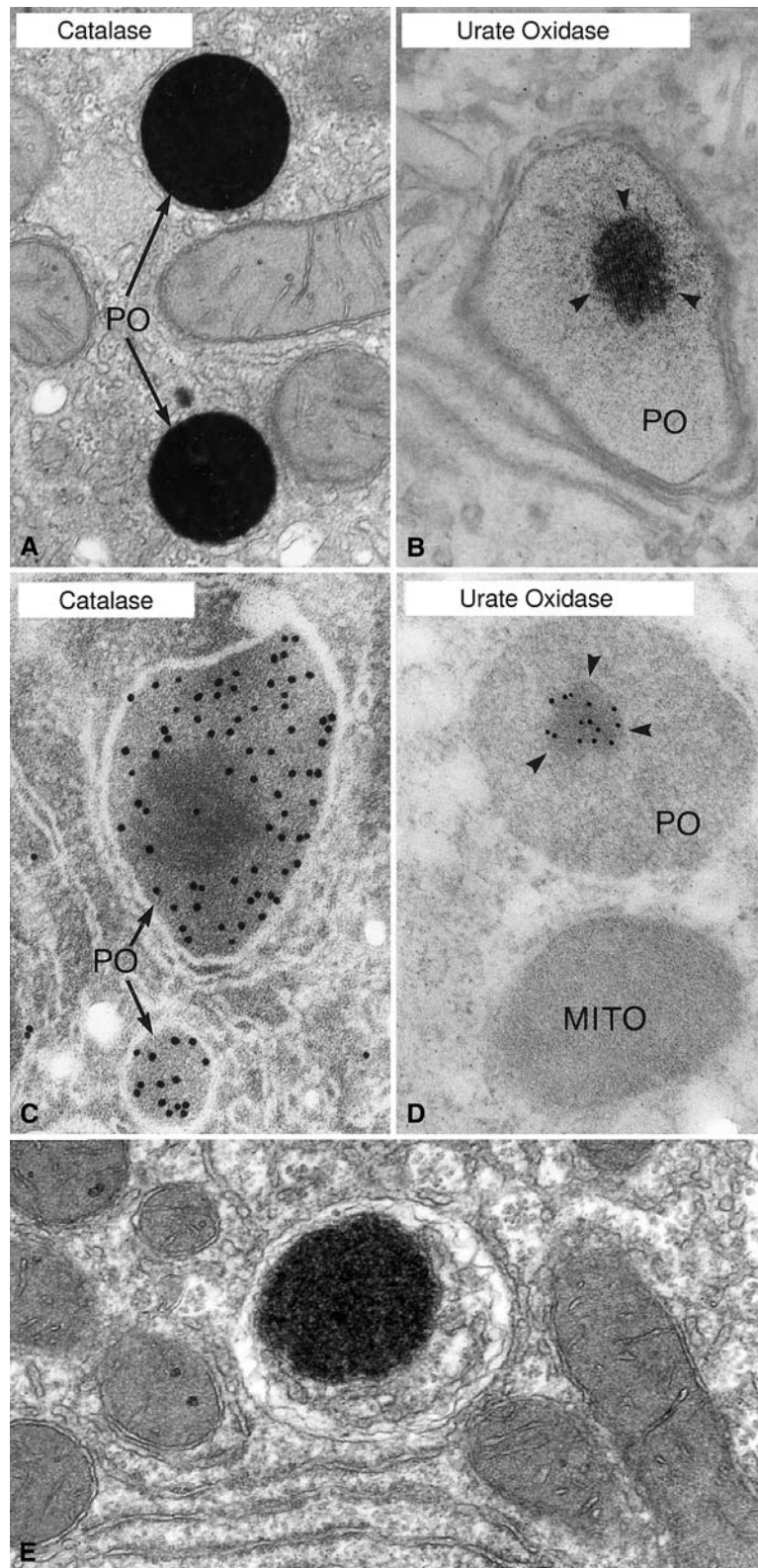
**Fig. 1** The major metabolic pathways in peroxisomes of the mammalian liver. The very-long-chain fatty acids (VLCFA) are transported by membrane proteins (e.g., the ABC transporter proteins PMP70 or ALDP) (see Table 1) into the matrix, where they are oxidized by the lipid  $\beta$ -oxidation enzymes. Multiple acyl-CoA oxidases and thiolases, as well as two distinct multifunctional (hydratase/3-hydroxyacyl-CoA dehydrogenase) enzymes coexist in peroxisomes (Poirier et al. 2006). The products of the  $\beta$ -oxidation can either serve as substrates for the biosynthesis of ether glycerolipids, cholesterol and bile acids or may exit the peroxisome for further oxidation in mitochondria (MITO). Peroxisomal  $\beta$ -oxidation and the activity of other peroxisomal oxidases result in the production of hydrogen peroxide, which is decomposed by catalase. Asterisks there are separate enzymes for bile acid intermediates, sER smooth endoplasmic reticulum, ALDP adrenoleukodystrophy protein

electron microscopy became available with the introduction of the alkaline 3, 3'-diaminobenzidine (DAB) reaction for catalase (Fahimi 1968, 1969; Novikoff and Goldfischer 1969) (Fig. 2). Subsequent morphological studies exploiting this cytochemical procedure revealed that peroxisomes, such as mitochondria, are ubiquitous eukaryotic organelles (Hruban et al. 1972). They disappear during the development of red blood cells and sperms (Luers et al. 2006), and appear to be absent in the Apicomplexa phylum and in amitochondriate parasites (Schluter et al. 2006) (see “Peroxisomes in silico”). In contrast to mitochondria, peroxisomes (0.1–1  $\mu\text{m}$  in diameter) are devoid of DNA, and have a single-limiting membrane surrounding a fine granular matrix, which may contain crystalline inclusions of matrix enzymes (Fig. 2). Pharmacological studies with hypolipidemic drugs and plasticizers (so-called peroxisome proliferators) led to the observation that peroxisomes can remarkably increase in number and size, especially in the livers of rodents (Hess et al. 1965; Svoboda and Azarnoff 1966; Fahimi et al. 1982; Reddy and Lalwani 1983). Such a

“peroxisome proliferation” is often accompanied by an increase in the synthesis of peroxisomal enzymes, and can result in the formation of hepatic tumors, mainly in rodents (Reddy et al. 1980, 1982; Moody et al. 1991). This selective induction of peroxisomal genes by those compounds is mediated by peroxisome proliferator activated receptor- $\alpha$  (PPAR $\alpha$ ), which belongs to the family of nuclear transcription factors (Issemann and Green 1990) and acts as heterodimeric partner with retinoid X receptor by binding to the peroxisome proliferator response elements (PPREs). On the basis of similar pharmacological studies, a fatty acid  $\beta$ -oxidation system in peroxisomes was discovered (Fig. 1), which coexists and cooperates with the mitochondrial fatty acid  $\beta$ -oxidation system in animal cells (Lazarow and De Duve 1976; Wanders 2000). Interestingly, in plant cells (Cooper and Beevers 1969) and in eukaryotic microorganisms, peroxisomes are the only site of  $\beta$ -oxidation (Poirier et al. 2006) which renders them essential for the utilization of fat in these organisms. Their important role in lipid metabolism as well as in health and disease became obvious in the 1980s, when the major function of peroxisomes in the  $\beta$ -oxidation of very-long-chain fatty acids (VLCFA) and in the biosynthesis of ether glycerolipids (plasmalogens) was discovered (Hajra et al. 1979; Brown et al. 1982) (Fig. 1). Their absence in Zellweger syndrome, the first genetic neurodegenerative peroxisomal disorder, was reported by Goldfischer et al. (1973) and was later linked to abnormalities in lipid metabolism (Heymans et al. 1983). Since then, ongoing research on the biogenesis of peroxisomes and their metabolic functions has greatly improved our knowledge about their crucial role in several inherited disorders, which are often not compatible with life or normal development (Weller et al. 2003; Wanders and Waterham 2005; Steinberg et al. 2006; Wanders and Waterham 2006a) and in other pathophysiological conditions (Table 1 and “Mysterious protection: some recent observations on the relevance of peroxisomes in medicine”).

About 85 genes in *Homo sapiens* and 61 genes in *Saccharomyces cerevisiae* have been identified, which encode for peroxisomal proteins. Many of these proteins are metabolic enzymes (about 50 in mammalian peroxisomes), whereas some 32 proteins/genes, so-called *peroxins* (Pex), have been discovered, which are required for the biogenesis and maintenance of functional peroxisomes (32 in yeast, with approximately 20 mammalian and 23 plant homologs) (Kiel et al. 2006; Platta and Erdmann 2007a) (Fig. 3). Besides their essential catabolic (oxidation of pipercolic, phytanic and very-long-chain fatty acids) and anabolic (synthesis of plasmalogens, bile acids and cholesterol) functions in lipid metabolism (Fig. 1), peroxisomes play a key role in free radical detoxification, differentiation, development and morphogenesis from human to yeast. Although many peroxisomal enzymes and metabolic pathways have been well characterized (Table 2),

**Fig. 2** **a** Cytochemical localization of catalase in rat hepatic peroxisomes stained with the alkaline diamino-benzidine technique (Fahimi 1969). Note the uniform staining of the peroxisome matrix. Magnification,  $\times 28,600$ . **b** Cytochemical localization of urate oxidase in rat liver using the cerium method (Angermuller and Fahimi 1986). Note the dark staining of the crystalline core (arrowheads). Magnification,  $\times 50,400$ . **c** Immunocytochemical localization of catalase in rat liver using the protein A-gold technique (Fahimi et al. 1996). Note the diffuse labelling of the matrix with gold particles and the sparing of the core region. Magnification,  $\times 72,000$ . **d** Immunocytochemical localization of urate oxidase in rat liver using the protein A-gold technique (Völkl et al. 1988). Note the exclusive labelling of the core with gold particles (arrowheads). Magnification,  $\times 61,600$ . **e** An autophagic vacuole in rat liver from an animal treated with the peroxisome proliferator bezafibrate. Note the presence of a peroxisome, stained for catalase with the diamino-benzidine reaction, in the autophagic vacuole. Magnification,  $\times 38,000$ . *PO* peroxisome, *MITO* mitochondrion



research on peroxisomal metabolism is still continuing (van den Bosch et al. 1992; Wanders and Waterham 2006b). Noteworthy, peroxisomes in plants, yeasts and protozoa generally possess a far wider spectrum of activities than in vertebrates

(e.g. penicillin biosynthesis in filamentous fungi, glyoxylate cycle, photorespiration, plant hormone biosynthesis/metabolism, and pathogen interaction in plants) (van der Klei et al. 2006; Kunze et al. 2006; Reumann and Weber 2006).

**Table 1** Disorders related to peroxisomes

Peroxisome biogenesis disorders			
Peroxisomal disease	Onset	Defect	Genes involved
Zellweger syndrome (ZS) (neonatal hypotonia, craniofacial dysmorphism, hepatomegaly, renal cysts, adrenal atrophy, neurological abnormalities, such as dys- or demyelination and neuronal migration defects)	Prenatal, lethal within 1 year of age	Peroxisome biogenesis	Different <i>PEX</i> genes
Neonatal ALD (NALD)	1 year, lethal between 1 and 5 years of age	Peroxisome biogenesis	Different <i>PEX</i> genes
Infantile Refsum's disease (IRD)	<1 year, lethal after 10–30 years of age; older patients are known	Peroxisome biogenesis	Different <i>PEX</i> genes
Rhizomelic chondrodysplasia punctata type 1 (RCDP type 1)	Prenatal, lethal within 1 year or the first decade; older patients are known	PTS2 matrix protein import	PEX7
Single enzyme deficiencies			
Peroxisomal disease	Onset	Enzyme and pathway affected	Genes involved
X-linked adrenoleukodystrophy (X-ALD) (accumulation of VLCFA, progressive demyelination/neurodegeneration in the CNS, adrenal insufficiency) "Lorenzos Oil"	3–10 years for childhood ALD, 20–40 years for adult AMN, lethal	ALDP, $\beta$ -oxidation of VLCFA	ABCD1
Contiguous ABCD1/DX1357E deletion syndrome	Prenatal	ABC transporters, $\beta$ -oxidation	ABCD1, BCAP31
Pseudo-neonatal ALD (acyl-CoA oxidase deficiency)	Prenatal, lethal within the first decade	ACOX1, $\beta$ -oxidation	ACOX1
D-bifunctional protein deficiency/multifunctional protein 2 deficiency	Prenatal	DBP, $\beta$ -oxidation	HSD17B4
Adult-onset sensory motor neuropathy ( $\alpha$ -methylacyl-CoA racemase deficiency)	Childhood and adult	AMACR, $\beta$ -oxidation of branched chain fatty acids, including pristanic acid and bile acid intermediates; $\beta$ -oxidation of VLCFA is normal	AMACR
Refsum's disease (phytanol-CoA hydroxylase deficiency)	10–20 years	PHYH/PAHX, $\alpha$ -oxidation	PHYH/PAHX
Rhizomelic chondrodysplasia punctata type 2 (DHAPAT deficiency)	<1 year	DHAPAT, ether phospho-lipid synthesis	GNPAT
Rhizomelic chondrodysplasia punctata type 3 (alkyl-DHAP synthase deficiency)	Prenatal	ADHAPS, $\beta$ -oxidation	AGPS
Sterol carrier protein X deficiency	1 patient	SCPx $\beta$ -oxidation of branched chain fatty acids only	SCP2
Hyperoxaluria type 1 (alanine glyoxylate aminotransferase deficiency)	<5 years, lethal	AGT, glyoxylate detoxification	AGXT
Acatalsasaemia	Adult	CAT, H <sub>2</sub> O <sub>2</sub> -metabolism	CAT
Potential other single enzyme deficiencies			
(Peroxisomal) disease	Onset	Enzyme and pathway affected	Genes involved
Mental retardation X-linked 63, MRX63	?	Long-chain acyl-CoA synthetase 4, fatty acid activation	FACL4
Malonic aciduria (MCD localizes to mitochondria, the cytosol and peroxisomes)	Early childhood (5 years)	Malonyl-CoA decarboxylase, fatty acid oxidation, but no peroxisomal abnormalities detected so far	MLYCD
Sjogren–Larsson syndrome	At birth or soon after	Aldehyde dehydrogenase, phytanic acid metabolism/microsomal detoxification system	ALDH3A2
Glutaryl-CoA oxidase deficiency (glutaric aciduria type 3)	1 year	Glutaryl-CoA oxidase	?
Amyotrophic lateral sclerosis (ALS1)	Variable (19–46 years)	Cu/Zn-superoxide dismutase 1	SOD1

**Table 1** continued

Potential other single enzyme deficiencies			
Xanthinuria (XDH localizes to peroxisomes and the cytosol)	Childhood (1–20 years)	Xanthine dehydrogenase, purine metabolism	XDH
Mulibrey nanism (muscle–liver–brain–eye nanism)	Perinatal	TRIM domain-dependent E3 ubiquitin ligase, unknown	TRIM37
Glomerulosclerosis (there is evidence that Mpv17p is mitochondrial and not peroxisomal; Spinazzola et al. 2006)	Only in mice	Mpv17 protein, PXMP 2/4 family proteins	MPV17
Combined mitochondrial peroxisomal deficiencies			
Disease	Onset	Enzyme and pathway affected	Genes involved
DLP1 deficiency (Waterham et al. 2007)	Prenatal, lethal	Dynammin-like GTPase, Fission defect of both mitochondria and peroxisomes	DNM1L

Mevalonate kinase deficiency is no longer considered to be a peroxisomal disorder (Hogenboom et al. 2004)

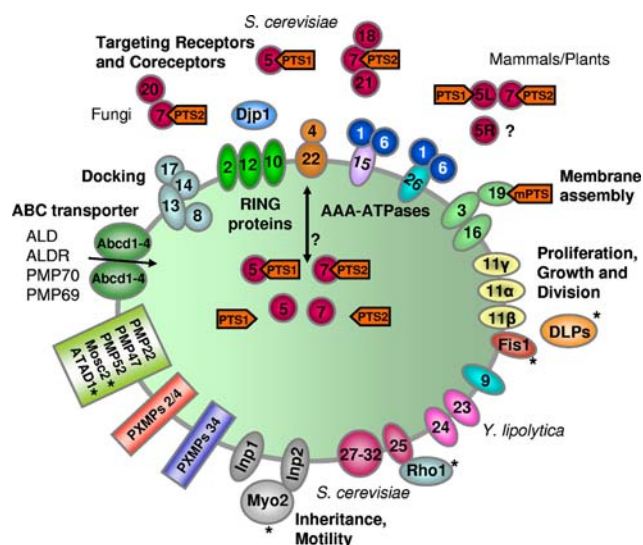
Peroxisomes are essential for human health and normal development. A defect in a peroxisomal gene can lead to a *single enzyme deficiency* which might affect one specific peroxisomal function or metabolic pathway. In *peroxisome biogenesis disorders* (PBDs) the affected protein is a peroxin (involved in the biogenesis and maintenance of peroxisomes). In PBDs several or all peroxisomal functions can be affected, and peroxisomes can be completely absent. As many peroxins are involved in matrix protein import (targeting, docking, translocation and receptor recycling) (see **Introduction** and Fig. 3), a lack of matrix protein import is often observed, whereas the synthesis of peroxisomal membranes and import of PMPs is unaffected. Loss of matrix protein import results in the formation of “empty”, non-functional peroxisomal membranes, so-called “ghosts”, which cannot fully develop and mature. The peroxisomal matrix proteins remain in the cytosol, where they cannot function or are degraded. An accumulation of peroxisomal substrates (e.g., VLCFA, plant-derived pristanic and phytanic acids, bile acid intermediates, and pipecolic acid, an intermediary in lysine metabolism) occurs, which can only be handled by peroxisomes, and are toxic for the cell/organism. Furthermore, a shortage of end products of peroxisomal metabolism (e.g., ether glycerolipids/plasmalogens, which comprise more than 80% of the phospholipid content of white matter in the brain) is observed. Organs affected in most peroxisomal disorders include brain, spinal cord, or peripheral nerves, eye, ear, liver, kidney, adrenal cortex, Leydig cells in testis, skeletal system, and in some instances cardiovascular system, thymus, and pancreas. Centres for the study of peroxisomal diseases are the Laboratory of Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands, and the Kennedy Krieger Institute, Baltimore, MD/USA. Links: The Myelin Project (<http://www.myelin.org/>), OMIM (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim>)

## The biogenesis of peroxisomes

### Protein import into peroxisomes: the mysterious membrane pore

Peroxisomes do not contain DNA or protein translation machinery, and all their proteins are encoded by nuclear genes. Majority of the peroxisomal proteins are synthesized on free polyribosomes in the cytoplasm and are imported post-translationally (Lazarow and Fujiki 1985) (Fig. 3). A major breakthrough in the elucidation of the mechanism of protein import into peroxisomes was the identification of the first peroxisomal targeting signal (PTS1) at the C-terminus of luciferase of the firefly *Photinus pyralis* (Keller et al. 1987; Gould et al. 1987). Luciferase actually localizes to peroxisomes in cells of the lantern organ of the firefly where it catalyzes the light-producing bioluminescent reaction. Today, we know that majority of the peroxisomal matrix proteins contain a C-terminal PTS1, and very few an N-terminal PTS2. It also became clear that the import into peroxisomes is a unique process, which differs substantially from the import mechanisms into the ER, mitochondria or chloroplasts. The peroxisomes import fully folded, co-factor bound and even oligomeric proteins by shuttling receptors (Leon et al. 2006). The PTS1- or PTS2-containing

matrix proteins are recognized by soluble receptors (PTS1 by Pex5p, a tetra-tricopeptide repeat (TPR) domain protein, PTS2 by Pex7p, a WD40 domain protein, and its coreceptors) in the cytosol, which guide them to a docking site at the peroxisomal membrane (Fig. 3). After translocation of the receptor–cargo complex to the luminal side of the peroxisomal membrane, the cargo is released and the receptors shuttle back to the cytosol. Whereas the components of the import machinery (the importomer complex; composed of the docking and RING subcomplexes which in yeast are bridged by Pex8p or Pex3p) (Fig. 3) and their interactions have been quite well studied (Rayapuram and Subramani 2006; Platta and Erdmann 2007b), the mechanism of translocation of folded proteins across the membrane and the cargo release still remain mysterious (Gould and Collins 2002). A stable import channel or membrane pore, like in the nucleus, is missing in the peroxisomal membrane. As an alternative to a pore, a pinocytosis-like mechanism has also been proposed (McNew and Goodman 1996). Recently, the existence of a “transient pore” has been postulated, which might be dynamically formed by the import receptors themselves (Erdmann and Schliebs 2005). In this respect, it is also noteworthy that very little is known about the membrane channels and transporters of peroxisomes, which are involved in membrane permeability and solute transfer of



**Fig. 3** Schematic overview of peroxins and other proteins at the peroxisomal membrane. Cargo proteins containing the peroxisomal targeting signals PTS1 or PTS2 bind to the corresponding receptors Pex5p or Pex7p and form receptor-cargo complexes. The Pex7p–cargo complex requires accessory factors for import (Pex5pL, a long isoform of Pex5p, in mammals and plants, Pex18p and Pex21p in *S. cerevisiae*, Pex20p in *Neurospora crassa*, *Yarrowia lipolytica*, and *Hansenula polymorpha*). The receptor–cargo complex in yeast and mammals interacts with a docking site (Pex13p, Pex14p and Pex 17p). It is unclear whether the receptors translocate fully into the matrix (“extended shuttle hypothesis”) or remain associated with the translocation complex (“shuttle hypothesis”). The docking and RING complexes (Pex2p, Pex10p, Pex12p) involved in translocation in yeast and mammals are bridged by Pex8p in *S. cerevisiae*. Recycling of cargo-released Pex5p to the cytosol requires Pex4p, an ubiquitin-conjugating enzyme that is bound to Pex22p, and the AAA-ATPases Pex1p and Pex6p. Pex6p binds to Pex15p in *S. cerevisiae* or to Pex26p in humans. The DnaJ-like protein Djp1p assists in matrix protein import. Membrane assembly and insertion of peroxisomal membrane proteins (PMPs) (containing an mPTS) depends on Pex19p, Pex3p and Pex16p. Pex19p functions as a cycling receptor/chaperone, which binds the PMPs in the cytosol and interacts with Pex3p at the peroxisomal membrane. Pex11p $\alpha$ , Pex11p $\beta$  and Pex11p $\gamma$  are the only peroxins known to be involved in the regulation of peroxisome size and number (proliferation) in mammals. In *Y. lipolytica* (Pex23p, and Pex24p) and *S. cerevisiae* (Pex25p, Pex27p–Pex32p) several other peroxins have been identified which influence the size and number or organization of peroxisomes. The division of peroxisomes is mediated by Fis1p and dynamin-like GTPases (DLP1/DRP1 in mammals, DRP3A in plants, Vps1p, Dnm1p in *S. cerevisiae*). Fis1p is the membrane receptor for DLP1 on peroxisomes (and mitochondria). Inp1p and Inp2p are involved in the inheritance and motility of peroxisomes in *S. cerevisiae* and *Y. lipolytica*. Inp2p is the membrane receptor for the type V myosin motor Myo2p on peroxisomes, which drives peroxisomes along actin filaments. The GTPase Rho1p binds to Pex25p and is involved in the recruitment of actin to peroxisomes in *S. cerevisiae*. Mammalian peroxisomes move along microtubules and bind dynein and kinesin motors by a yet unknown mechanism. Other membrane proteins are members of the PXMP34 and PXMP2/4 families, as well as the four ABC transporter proteins in mammals. Other PMPs are PMP22, PMP47, and the recently identified PMP52, as well as Msc2 and ATAD1, an AAA-ATPase. Proteins with a dual localization to both peroxisomes and mitochondria are marked with *asterisk*. The ORF of *YIPEX9* was misidentified; it corresponds to *HsPEX26*. *ALD* adrenoleukodystrophy protein, *ALDR* ALD-related protein

metabolites (Antonov and Hiltunen 2006; Visser et al. 2007).

It has also been shown that Pex5p can be ubiquitinated (Erdmann and Schliebs 2005; Kragt et al. 2005b; Thoms and Erdmann 2006). Monoubiquitination of Pex5p appears to be a prerequisite for its dislocation from the peroxisomal membrane, and depends on the E2 enzyme Pex4p (Platta and Erdmann 2007a; Carvalho et al. 2007). Its function has been a mystery for over a decade; however, respective E3 enzymes are still elusive (the RING finger proteins Pex2p, Pex10p and Pex12p might be good candidates for the missing ubiquitin ligases). The membrane detachment/recycling of Pex5p is ATP dependent (but not the binding and translocation of Pex5p itself), and depends on AAA-ATPases Pex1p and Pex6p (Fig. 3). Polyubiquitination of Pex5p is believed to be part of a quality control process, which results in proteasomal degradation of dysfunctional receptor molecules (Platta and Erdmann 2007a). Noteworthy, these processes show a striking analogy to ERAD, the ER-associated degradation process (see also “*Peroxisomes in silico*”).

Interestingly, there is experimental evidence that manipulation of the peroxisomal import of catalase has an influence on aging. The alteration of the PTS1 of catalase to a more effective serine–lysine–leucine (SKL) sequence repolarized mitochondria and reduced cellular hydrogen peroxide levels and the number of senescent cells in a population (Terlecky et al. 2006; Koepke et al. 2007). Furthermore, targeting of catalase to mitochondria in transgenic mice has demonstrated to increase murine life span (Schriner et al. 2005).

The targeting and insertion of peroxisomal membrane proteins (PMPs) require other components than those involved in peroxisomal matrix import and is less well understood (Heiland and Erdmann 2005; Van Ael and Fransen 2006; Fujiki et al. 2006). Those PMPs, which are synthesized on free ribosomes in the cytosol, contain internal membrane targeting sequences (mPTS), which comprise a Pex19p-binding site and a membrane-anchoring sequence (either a transmembrane domain or a protein binding site) (Van Ael and Fransen 2006). Pex19p is suggested to function as a cycling receptor/chaperone, which binds the PMPs in the cytosol and is then recruited to the peroxisome by the membrane receptor Pex3p (Fujiki et al. 2006) (Fig. 3). In mammals, Pex16p is also required, which might function as a tethering factor for Pex3p, or as part of the putative membrane-insertion machinery. A loss of Pex3p, Pex16p, or Pex19p results in the absence of detectable peroxisomes/peroxisomal membranes, whereas reintroduction surprisingly leads to a de novo formation of peroxisomes from the ER (see below). Some PMPs (e.g., Pex3p and Pex16p) are supposed to be targeted indirectly to peroxisomes via the ER by an as yet unknown mechanism (see below).

**Table 2** Metabolic functions of peroxisomes

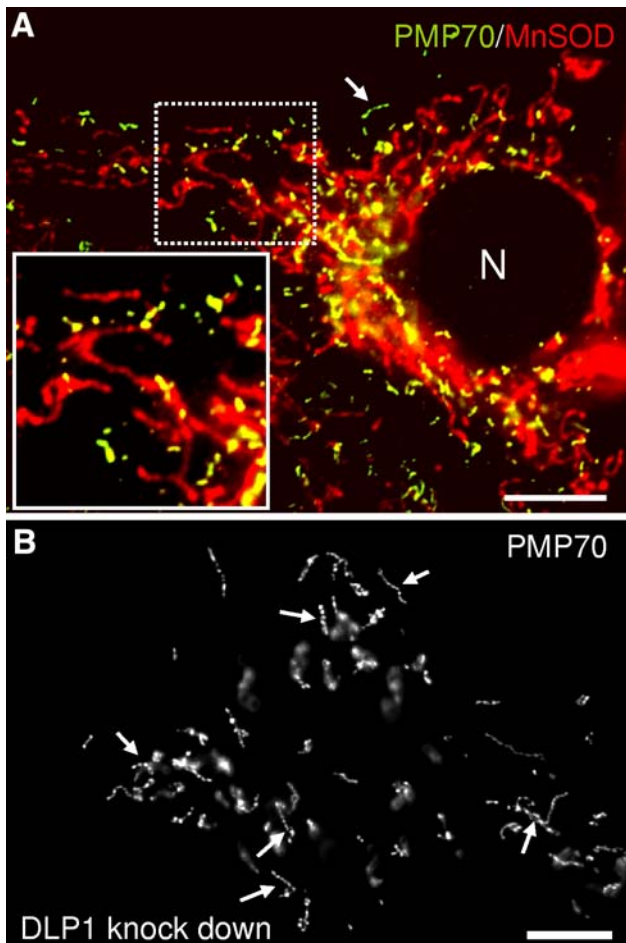
Peroxide metabolism (catalase and H <sub>2</sub> O <sub>2</sub> -generating oxidases), ROS/NOS metabolism
Lipid biosynthesis (ether phospholipids/plasmalogens, bile acids, cholesterol and dolichol, fatty acid elongation)
Fatty acid $\beta$ -oxidation (very-long-chain fatty acids, dicarboxylic acids, branched chain fatty acids, unsaturated fatty acids, arachidonic acid metabolism, and xenobiotic compounds)
Fatty acid $\alpha$ -oxidation (phytanic acid, xenobiotic compounds)
Long/very-long fatty acid activation
Regulation of acyl-CoA/CoA ratio
Protein/amino acid metabolism (biosynthesis of cysteine and sulfur assimilation, D-amino acid degradation, L-lysine metabolism, degradation of polyamines, proteases, transaminases)
Catabolism of purines
Glyoxylate and dicarboxylate metabolism
Hexose monophosphate pathway
Glycerol synthesis
Nicotinate and nicotinamide metabolism
Retinoid metabolism

Mysterious formation: growth and division, de novo formation, or both?

Since their discovery in 1954, there has been much speculation about the formation of peroxisomes. It was suggested that peroxisomes were precursors of mitochondria (Bernhard and Rouiller 1956), that they could arise from the Golgi complex (Rouiller and Jezequel 1963) or were specialized forms of lysosomes (Novikoff and Essner 1960). Whereas those concepts were abandoned with improvements in cytochemical methods, it was also proposed that peroxisomes formed from terminal cisternae of the ER (Novikoff and Shin 1964). However, the discovery of the synthesis of peroxisomal proteins on free polyribosomes in the cytoplasm (Goldman and Blobel 1978), their post-translational transport into peroxisomes, and the observations of interconnections between peroxisomes, led to the proposal of the “growth and division” model of peroxisome biogenesis with formation out of pre-existing organelles (Lazarow and Fujiki 1985). Recent discoveries have again challenged this classical view. The peroxins Pex3p, Pex19p and Pex16p are required to maintain the peroxisomal membrane (South and Gould 1999; Hettema et al. 2000; Heiland and Erdmann 2005), and their loss of function leads to the absence of peroxisomal membranes and thus, peroxisomes. Transexpression of Pex3, Pex19 or Pex16 and re-introduction of the missing genes has now been demonstrated to restore peroxisome formation in yeast and mammals, and lead to the postulation of a de novo synthesis of peroxisomes (Matsuzono et al. 1999; South and Gould 1999; Muntau et al. 2000; Titorenko and Rachubinski 2001; Faber et al. 2002; Geuze et al. 2003; Hoepfner et al. 2005; Kragt et al. 2005a; Haan et al. 2006; Kim et al. 2006). Pex3p and Pex19p have been observed to initially localize

to the ER before maturing into import-competent peroxisomes (Hoepfner et al. 2005; Tam et al. 2005; Haan et al. 2006), indicating that the ER/nuclear envelope is the source of the newly synthesized membrane and organelle.

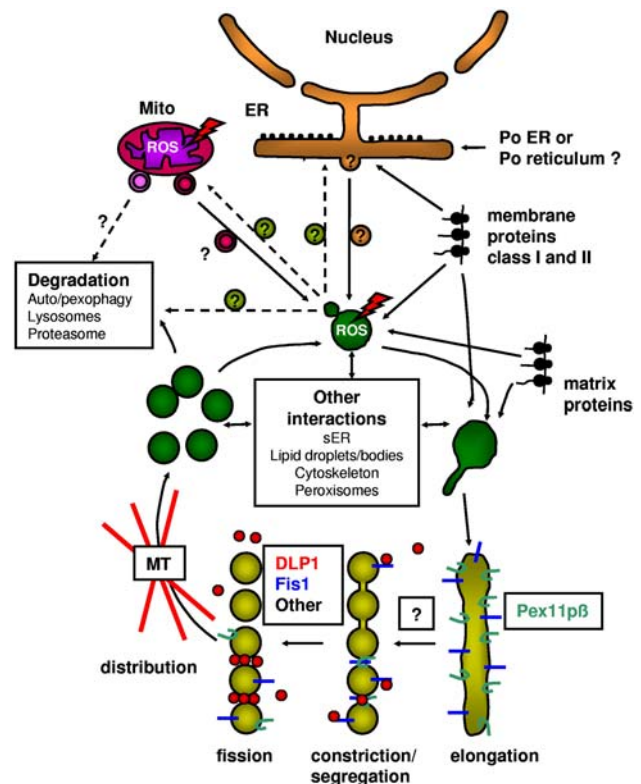
However, peroxisomes have also been reported to divide, and recently proteins have been identified in yeast, mammalian and plant cells, which play a role in peroxisomal fission. Among them are dynamin-like GTPases (DLPs) with mechanochemical properties (yeast Dnm1p and Vps1p, mammalian DLP1/Drp1, plant DRP3A), Fis1p, a membrane adaptor for DLPs, and Pex11 proteins (for recent reviews see Schrader and Fahimi 2006; Fagarasanu et al. 2007) (Fig. 3). Morphological observations show that growth and division of peroxisomes in mammalian cells is a multistep process including peroxisome elongation, constriction, and final fission (Koch et al. 2003, 2004, 2005) (Figs. 4, 5). Peroxisomes tend to have a characteristic, segmented, “beads on a string”-like appearance before they divide and distribute (Schrader et al. 1996), or when DLPs are non-functional (Hoepfner et al. 2001; Koch et al. 2003, 2004; Tanaka et al. 2006; Waterham et al. 2007) (Fig. 4). Pex11 proteins are implicated early on in the elongation step of peroxisomes, whereas Fis1p and DLPs are required for the final fission step (Thoms and Erdmann 2005; Schrader and Fahimi 2006; Fagarasanu et al. 2007; Kobayashi et al. 2007) (Fig. 5). Peroxisomal constriction can proceed independently of DLP1 (Koch et al. 2004), but the molecular mechanism is so far largely unknown. Interestingly, Fis1p and DLP1 (Dnm1p, DRP3A) are also involved in mitochondrial fission (Hoopes et al. 2007), and are shared by both organelles (Schrader 2006). These findings indicate that peroxisomes and mitochondria share some basic characteristics and are much closer than previously assumed, thus underlining the tight cooperation and cross talk between both organelles



**Fig. 4** Fluorescence microscopy of peroxisomes (**a, b**) and mitochondria (**a**) in human hepatoma cells. Cells were stained with antibodies to the peroxisomal membrane protein PMP70 (**a, green; b**) and to mitochondrial manganese superoxide dismutase (MnSOD) (**a, red**). Peroxisomes exhibit spherical, rod-shaped and elongated, tubular morphologies, whereas mitochondria form an interconnected tubular network. During division peroxisomes appear like “beads on a string” (*arrow*) (Schrader et al. 1996). *Boxed region* shows higher magnification view. (**b**) Peroxisome morphology after silencing of DLP1 by siRNA. Note the highly elongated but constricted appearance of peroxisomes defective in final membrane fission (*arrows*). The formation of spherical peroxisomes by division is completely inhibited. *N* nucleus. *Scale bars*, 10  $\mu\text{m}$  (**a, b**)

(Schrader and Yoon 2007) (Figs. 4, 5; and “Peroxisomes and mass spectrometry”). Similar to mitochondria, peroxisomal morphology and dynamics might influence peroxisomal functions as well as developmental and physiological processes. Interestingly, a lethal defect in peroxisomal and mitochondrial fission, which appears to be based on a point mutation in the DLP1 gene, has recently been described (Waterham et al. 2007). These findings might point to a new class of diseases characterized by defects in peroxisomes and mitochondria (Table 1).

The physiological significance of the mechanism of de novo formation in comparison to the classical pathway of



**Fig. 5** Model of peroxisome dynamics and interactions in mammalian cells. The majority of the peroxisomal matrix and membrane proteins (Class I PMPs) are synthesized on free polyribosomes in the cytosol and are post-translationally imported into pre-existing peroxisomes. Some membrane proteins (Class II PMPs, *early peroxins*, for example, Pex3p) are routed to peroxisomes via the ER or a pre-peroxisomal compartment, presumably by vesicular transport. A retrograde peroxisome-to-ER transport might exist. A novel vesicular mitochondria-to-peroxisome trafficking route has been described recently (Neuspiel et al. 2008), but its function is still mysterious. It is currently unknown, whether a retrograde peroxisome-to-mitochondrion transport exists, and whether peroxisome-derived vesicles contribute to the removal of damaged (e.g., by ROS production), misfolded or mistargeted proteins. Besides ER and mitochondria (Mito), peroxisomes are supposed to interact with the smooth ER (sER), with lipid droplets/bodies, the cytoskeleton, and other peroxisomes (Schrader et al. 2000). Peroxisomes can multiply by growth/elongation, constriction and final fission/division, forming spherical peroxisomes. Pex11p $\beta$  is involved in the elongation/tubulation of peroxisomes, whereas DLP1/Drp1 and Fis1p mediate peroxisomal fission (Schrader and Fahimi 2006). Fis1p is supposed to recruit cytosolic DLP1/Drp1 to the peroxisomal membrane. The involvement of other proteins (related to yeast Mdv1p/Caf4p) is likely. Components involved in the constriction of peroxisomes are presently unknown. Proper intracellular distribution of the peroxisomes formed by fission requires microtubules (MT) and motor proteins. In yeast and plants peroxisomes are distributed via the actin cytoskeleton. The consecutive steps are linked to each other, and may be triggered by the assembly of distinct machineries at the peroxisomal membrane

growth and division is still controversially discussed (Lazarow 2003; Kunau 2005; Haan et al. 2006; Mullen and Trelease 2006; Tabak et al. 2006). In order to investigate to what extent these pathways of peroxisome formation



contribute to peroxisome numbers in yeast, Motley and Hetteima (2007) have developed an elegant, fluorescence-based mating assay to follow the fate of existing and de novo-formed peroxisomes in *S. cerevisiae*. They provide evidence that in wild-type cells (grown on a nonfermentable carbon source) peroxisomes multiply by growth and division and do not form de novo. Only cells lacking peroxisomes as a result of a segregation defect were observed to form peroxisomes de novo out of the ER. In contrast to peroxisome fission, de novo formation was much slower and appeared to be independent of DLPs such as Dnm1p or Vps1p (Motley and Hetteima 2007; Nagotu et al. 2007; Jourdain et al. 2008). In some yeasts, which possess only a few peroxisomes, de novo formation may therefore represent a rescue mechanism that becomes functional in case peroxisomes are lost (e.g., due to failure in inheritance). Several components involved in peroxisome inheritance in yeast have recently been discovered, for example the peroxisomal proteins Inp1p and Inp2p, which together with the type V myosin motor Myo2p and actin play a role in the retention and motility of peroxisomes (Fagarasanu et al. 2006, 2007) (Fig. 3).

However, there is now also firm evidence that some peroxisomal proteins are routed indirectly to peroxisomes via the ER (e.g. Pex3p) (Hoepfner et al. 2005; Tam et al. 2005; Kragt et al. 2005a; Haan et al. 2006; Kim et al. 2006; Mullen and Trelease 2006; Motley and Hetteima 2007). This trafficking pathway is supposed to involve ER-derived vesicular or preperoxisomal structures, which do not require DLPs for exit from the ER (Motley and Hetteima 2007; Nagotu et al. 2007), and are observed to fuse with pre-existing peroxisomes. Thus, a semi-autonomous model of peroxisome formation can be envisaged, whereby the ER supplies existing peroxisomes with essential membrane proteins (and lipids) to allow peroxisomal growth and division (Fig. 5). However, it is currently not understood how the peroxisomal proteins enter and leave the ER, how they are sorted and packaged, what the nature and composition of the ER-derived structures is, how fusion with peroxisomes is mediated, and if a retrograde, peroxisome-to-ER pathway exists (Fig. 5). Interestingly, such a retrograde transport pathway has recently been proposed in infected plant cells (McCartney et al. 2005; Mullen and Trelease 2006) and trypanosomes (Subramanya and Mensa-Wilmot 2006). Furthermore, a role of the Dsl1p secretory complex in regulating de novo synthesis of peroxisomes from the ER has been very recently proposed in *S. cerevisiae* (Perry and Rachubinski 2007).

Mysterious trafficking: a novel vesicular mitochondria-to-peroxisome pathway?

Besides vesicular ER-to peroxisome transport, even a transport route between mitochondria and peroxisomes might exist (Fig. 5). Neuspiel et al. (2008) have very recently iden-

tified a new outer membrane mitochondrial anchored protein ligase (MAPL) containing a RING finger domain. MAPL appeared to have a regulatory function in controlling mitochondrial morphology. Excitingly, MAPL was also incorporated within unique, vesicular structures, which emanated from the sides of mitochondria. These new mitochondrial-derived vesicles (MDVs) (70–100 nm in diameter) formed in a DLP1-independent manner, showed an increase in electron density around the surface, and often contained both outer and inner mitochondrial membranes, which appeared as two concentric circles. Remarkably, in live-cell experiments MAPL-YFP-positive vesicles were observed to fuse with a small subset of peroxisomes (Neuspiel et al. 2008). Furthermore, other populations of MDVs were discovered, which excluded MAPL, but contained TOM20 (which was absent from MAPL-positive MDVs). The TOM20-positive MDVs did not fuse with peroxisomes, and their fate is still under investigation. However, it might be possible that these vesicles contribute to the removal of damaged, misfolded, or mistargeted proteins from the mitochondria/mitochondrial membranes, and route them to the lysosomal/autophagic degradation pathway (Fig. 5). The physiological function of the new vesicular mitochondria-to-peroxisome transport pathway is also still mysterious. MAPL appears to be present at low levels in only a subpopulation of peroxisomes, and is not required for MDV formation at the mitochondria or targeting to peroxisomes. Silencing or overexpression of MAPL was not observed to change peroxisomal morphology, and thus, it might not play a role in peroxisomal growth and division (in contrast to its function on mitochondria). Peroxisomes and mitochondria are metabolically linked organelles, which cross talk and cooperate, and even share components of their division machinery (Schrader 2006; Schrader and Yoon 2007) (Figs. 1, 4). The MAPL-positive MDVs might therefore function in the transport of metabolites, lipids, or proteins to a peroxisome subpopulation. Interestingly, a dual localization to both peroxisomes and mitochondria has been observed for mitochondrial enzymes and some mitochondrial membrane proteins (e.g., Fis1p, Mosc2, and ATAD1, a member of the AAA-superfamily of ATPases) (Koch et al. 2005; Wiese et al. 2007) (see “[Peroxisomes and mass spectrometry](#)”). However, Fis1p and the other proteins are found in the vast majority of peroxisomes (distinct from MAPL), and are presumably imported directly from the cytosol. An alternative function of the peroxisome-targeted MDVs might be the retrieval of peroxisomal (membrane) proteins, which have been mistargeted to mitochondria. Mistargeting of peroxisomal membrane proteins to mitochondria is often observed under different experimental conditions, for example when peroxisomal membrane insertion is affected (Sacksteder et al. 2000; Soukupova et al. 1999), and might be due to some overlap in the targeting information that is also recognized by the mitochondrial import machinery (Van Ael

and Fransen 2006; Subramani 1998). Another interesting issue is the fusion event of a double-membrane-bound MDV with a single-membrane-bound peroxisome (Neuspiel et al. 2008). Such a fusion would likely result in the release of a single-membrane-bound vesicle (the former inner mitochondrial membrane) into the lumen of the peroxisome. Internal peroxisomal membrane structures have been described in yeast cells and in mammalian tissue (McNew and Goodman 1996), and very recently after deletion of the peroxisomal lipase Lpx1p in *S. cerevisiae* (Thoms et al. 2008). It will be very interesting to elucidate the fate of the putative vesicle remnant, and to identify the components of the putative MDV-peroxisome fusion machinery.

It is intriguing to speculate that peroxisomes themselves—like mitochondria—might be able to emanate vesicles from their membranes (which would then be PDVs, peroxisome-derived-vesicles) (Fig. 5). Budding events at the peroxisomal membrane have been occasionally observed (Jedd and Chua 2002). Furthermore, ADP-ribosylation factor (ARF) and coatamer have been localized to peroxisomes (Passreiter et al. 1998; Lay et al. 2005). Efforts to link ARF and coatamer recruitment to (ER-derived) peroxisome formation or growth and division have so far been inconclusive or are difficult to interpret (Lay et al. 2006). It might actually be possible that these components play only a minor, if any, role in peroxisome formation, but are instead involved in the generation of PDVs, which might either deliver metabolites, lipids, or (mistargeted) proteins to mitochondria (or to other subcellular compartments, e.g., to the ER; see “Mysterious formation: growth and division, de novo formation, or both?”), or might be involved in the removal of damaged (e.g., by peroxisomal ROS production), misfolded, or mistargeted (mitochondrial?) proteins from the peroxisomal membranes, and route them to the lysosomal/autophagic degradation pathway (Fig. 5). As the peroxisome appears to be connected to transport pathways coming from the ER and presumably also from mitochondria, there will certainly be some degree of mistargeting. Therefore, mechanisms must exist, which assist the peroxisome in getting rid of potentially mistargeted proteins, especially those in the peroxisomal membrane, which are not readily accessible to peroxisomal enzymes/proteases (e.g., membrane proteins mainly exposed to the cytoplasm). Besides vesicle formation, these mechanisms might also include polyubiquitination (Platta and Erdmann 2007a) or autophagy/pexophagy of the whole organelle (Sakai et al. 2006) (see below).

### Mysterious removal: degradation and autophagy of peroxisomes

The number of peroxisomes in cells and their enzymatic equipment is modified depending on metabolic state and

the environmental requirements. A well-known characteristic of peroxisomes is their inducibility by divergent chemicals and drugs which is mediated by the transcription factors referred to as “peroxisome proliferator activated receptors” (PPARs) (Issemann and Green 1990). Upon withdrawal of treatment, the peroxisome proliferation is reversed and the excess particles are removed by autophagy (Yokota 1993) (Fig. 2e), which in the case of yeast cells is also referred to as *pexophagy* (Sakai et al. 2006). Autophagy is a process regulated by ATG genes, which determine the sequestration and degradation of cell organelles and other cellular components within lysosomes or vacuoles. Two distinct mechanisms of sequestration by autophagy have been described: *macroautophagy* and *microautophagy*. Whereas in macroautophagy the cell components (e.g., peroxisomes) are first sequestered within autophagosomes, which in turn fuse with lysosomes/vacuoles, in *microautophagy* membrane events occur on the surface of the vacuole/lysosome which then engulf the cell components to be digested resulting in the formation of microautophagic bodies (Sakai et al. 1998a). From the numerous ATG genes (Klionsky et al. 2003) involved in general cellular autophagy, many have also been identified to participate in pexophagy (Schroder et al. 2007). On the other hand, much less is known about the peroxisomal components, which are involved in this process. In the yeast *Hansenula polymorpha* two peroxisomal membrane proteins, HpPex3p (Bellu et al. 2002) and HpPex14p (Bellu et al. 2001), appear to be important in the process of macropexophagy. In yeasts lacking HpPex14p, degradation of peroxisomal membranes is defective and it is suggested that the highly conserved N-terminal region of HpPex14p is necessary for macropexophagy. Since Pex14p has also been known to be a translocon and is involved in the formation of peroxisomes (Fig. 3), it is the only Pex protein that participates both in peroxisome formation and degradation (Zutphen et al. 2008). The yeast models *Pichia pastoris* and *H. polymorpha* have extensively been used for the analysis of molecular events and the morphological steps of pexophagy, and there are excellent recent reviews on this subject (e.g., Dunn et al. 2005; Sakai et al. 1998b, 2006; Platta and Erdmann 2007a).

In mammalian hepatocytes, the degradation of peroxisomes by autophagy was noted quite early by electron microscopic studies in rodents treated with hypolipidemic drugs (Leighton et al. 1975; Moody and Reddy 1976; Staubli et al. 1977) (Fig. 2e). Although the morphological aspects and the participation of lysosomes in this process were well characterized (Yokota 2003), its molecular aspects remained quite mysterious compared to yeast cells. Recently, Iwata et al. (2006) reported that in Atg7-deficient mice (lacking the essential gene for autophagy), the excess peroxisomes induced by phthalate ester could not be

degraded within 1 week after discontinuation of the treatment. Moreover, the Atg7-deficient hepatocytes lacked the autophagosomes containing sequestered peroxisomes, thus confirming the role of autophagy in degradation of excess peroxisomes. Further studies are required to analyse the role of other genes and their products in autophagy in mammalian systems and the search has just started (Monastyrska and Klionsky 2006).

In addition to the autophagic degradation of excess peroxisomes discussed above, another unique aspect of turnover of this organelle deserves to be mentioned, namely, the possible role of 15-lipoxygenase in selective destruction of peroxisomal membranes in normal untreated animals. In reticulocytes, 15-lipoxygenase has been shown to bind selectively to the membranes of organelles and induce the diffusion of their contents (van Leyen et al. 1998). The importance of this process for the degradation of cell organelles in differentiating lens fibres (Bassnett and Mataic 1997) and in maturation of reticulocytes has been suggested (Schewe et al. 1975). This notion has been confirmed recently in Atg5<sup>-/-</sup> mice which show normal organelle degradation in lens fibres and erythroid cells in spite of inability to form autophagic vacuoles (Matsui et al. 2006). The presence of 15-lipoxygenase in the membranes of normal rat hepatocyte peroxisomes was demonstrated recently, and it was suggested that, similar to maturing reticulocytes and lens fibres, it could also be involved in the degradation of peroxisome membranes (Yokota et al. 2001). This notion is supported by the observation that 15-lipoxygenase was detected in only some but not all peroxisomes and that its presence correlated with the diffusion of catalase from some but not all peroxisomes in aldehyde fixed sections of rat liver. The latter finding was reported almost 30 years before and was contributed to the heterogeneous stability of peroxisome membranes (Fahimi 1974). Since the disruption of peroxisome membranes and the diffusion of catalase were both prevented by inhibitors of 15-lipoxygenase such as propyl gallate and esculetin, it was suggested that 15-lipoxygenase could participate in physiological degradation and turnover of peroxisomes in normal untreated animals, contrasting the role of autophagy in degradation of excess peroxisomes induced by treatment with chemicals and drugs (Yokota et al. 2001).

### **Mysterious protection: some recent observations on the relevance of peroxisomes in medicine**

There are by now more than two dozens inborn metabolic disorders due to various dysfunctions or absence of peroxisomes and that number is growing (Schluter et al. 2007). Table 1 summarizes those disease conditions with the age of the onset, the basic metabolic defect and the genes

involved. In addition to those genetic peroxisomal disorders, which have been addressed in recent excellent reviews (Weller et al. 2003; Wanders and Waterham 2005, 2006a; Faust et al. 2005; Steinberg et al. 2006), there is also increasing evidence that peroxisomes may be affected in some medical conditions associated with disorders of the lipid metabolism. Since peroxisomes and their proteins can be induced by a variety of drugs and chemicals, generally referred to as PPAR-agonists, there are emerging new possibilities for therapy of those conditions. Two such disorders are briefly discussed here: fatty liver disease and neuroinflammation.

### **Peroxisomes in fatty liver disease**

The fatty liver disease (FLD) represents an excess accumulation of triglycerides in hepatic parenchymal cells. Whereas in the past excess ethanol consumption accounted for most cases, which were called alcoholic FLD (AFLD), in the last two decades, the non-alcoholic FLD (NAFLD), particularly in association with obesity, has emerged as the most common chronic liver condition in the western world (Adams et al. 2005). NAFLD is now present in 17–33% of the population in the United States, but has a world-wide distribution, and parallels the frequency of central adiposity, obesity, insulin resistance, metabolic syndrome and type 2 diabetes (Farrell and Larter 2006). In almost one-third of NAFLD cases, there is an associated steatohepatitis (NASH), which progresses to hepatic fibrosis and upon chronicity to liver cirrhosis. Many cases of the so-called cryptogenic liver cirrhosis are likely end-stage NASH (Neuschwander-Tetri and Caldwell 2003). Although insulin resistance is the underlying problem, in the pathogenesis of FLD both excess energy consumption as well as defective energy combustion contribute to a sequence of events which begins with hepatic steatosis and ends in liver cirrhosis and cancer (Reddy and Rao 2006). Whereas hepatic steatosis is considered to be innocuous and reversible, the progression to steatohepatitis is influenced by the severity and persistence of the causative agent and additional factors. According to the “two-hit” hypothesis, secondary insults that may severely damage the steatotic liver include reactive oxygen species, endotoxin, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and other cytokines (Day and James 1998). In this respect, it should be noted that inflammatory cytokines such as TNF $\alpha$  have been shown to suppress the hepatic peroxisomal catalase and the lipid  $\beta$ -oxidation enzymes, as well as the transcription factor PPAR $\alpha$ , at both protein and mRNA levels (Beier et al. 1992, 1997). The reduction of the fatty acid  $\beta$ -oxidation enzymes in peroxisomes could contribute to the lipid accumulation in hepatocytes and to the severity of steatohepatitis. Indeed, mice lacking the peroxisomal acyl-CoA oxidase gene develop

severe steatohepatitis, lipogranulomas and hepatocellular carcinomas (Fan et al. 1998). Some of the features of those AOX<sup>-/-</sup> mice resemble the spectrum of obesity associated human liver alterations justifying further detailed studies of peroxisomes in patients with morbid obesity and type 2 diabetes.

From the PPAR subfamily of nuclear transcription factors (Michalik et al. 2006), the PPAR $\alpha$  functions mainly as a lipid sensor in the liver responding to the influx of fatty acids by up-regulation of the transcription of genes involved in  $\beta$ -oxidation of fatty acids in peroxisomes and mitochondria, as well as the microsomal  $\omega$ -oxidation system. PPAR $\alpha$ <sup>-/-</sup> mice cannot respond to increased fatty acid influx and develop severe hepatic steatosis (Hashimoto et al. 2000). Such PPAR $\alpha$ <sup>-/-</sup> mice also develop severe steatohepatitis after feeding with a choline and methionine deficient diet (Kashireddy and Rao 2004). Interestingly, both agonists of PPAR $\alpha$  and PPAR $\delta$  have been found to improve the hepatic steatosis and prevent the inflammation associated with steatohepatitis induced by choline-methionine deficient diet in mice (Nagasawa et al. 2006). More importantly, in rats with severe steatohepatitis and liver fibrosis induced by a choline-methionine deficient diet, administration of PPAR $\alpha$  agonists prevents not only the development of steatohepatitis but also reverses the fibrotic process in the liver (Rao et al. 2002). Thus, it seems that induction of fatty acid oxidation enzymes by activation of PPAR $\alpha$  and PPAR $\delta$  can prevent the development of hepatic steatosis. Indeed, in clinical studies, the PPAR $\gamma$  agonists rosiglitazone (Neuschwander-Tetri et al. 2003) and pioglitazone (Promrat et al. 2004) have been tried and found to improve significantly all histological and biochemical features of non-alcoholic steatohepatitis. This has also been proven in a placebo-controlled trial with pioglitazone justifying further long-term studies (Belfort et al. 2006). Thus, PPAR-agonists such as fibrates or thiazolidinediones offer the potential for treatment of not only the hepatic complications but also the underlying disease process of metabolic syndrome and type 2 diabetes with dyslipidaemia (Plutzky 2007; Staels 2007).

#### Peroxisomes and neuroinflammatory diseases

The severe structural and functional abnormalities of the central nervous system (CNS) seen in patients with peroxisome biogenesis disorders (PBDs) (Evrard et al. 1978; Powers et al. 1985) (Table 1) demonstrate the important role of peroxisomes in normal development and function of the brain. This is also confirmed in brain specific gene-knock out mouse models of PEX5 (Janssen et al. 2003) and PEX2 (Faust 2003), which exhibit neuronal migration defects and abnormalities in cerebellar histogenesis, respectively. Recently, in a mouse model of selective knock out of

PEX5 in oligodendrocytes, the central role of peroxisomes in the myelination process was demonstrated (Kassmann et al. 2007). Although the mutant mice appeared normal at birth, within a few months they developed ataxia, tremor and died prematurely with widespread evidence of axonal degeneration and progressive subcortical demyelination. Moreover, severe inflammation accompanied the axonal degeneration with infiltration of B and CD8+ T cells in cerebral lesions resembling closely the neuroinflammatory lesions associated with human demyelinating disorders, particularly the multiple sclerosis.

Thus, it seems that peroxisomes in oligodendrocytes have a neuroprotective function preventing not only the axonal degeneration but also the associated process of neuroinflammation. The anti-inflammatory function of peroxisomes and the regulatory role of PPAR-activators in this process were discovered more than a decade ago (Devchand et al. 1996). Both, PPAR $\alpha$  and PPAR $\gamma$ , are expressed on T cells, and their respective agonists inhibit the secretion of interleukin-2 and the proliferation of T cells (Cunard et al. 2002; Marx et al. 2002). Moreover, PPAR-agonists have been found to inhibit the progression of a variety of inflammatory diseases in experimental animals such as adjuvant arthritis in rats (Kawahito et al. 2000), inflammatory bowel disease (Su et al. 1999) and atherosclerosis in mice (Li et al. 2000). Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disorder in mice characterized by inflammation of the CNS and severe demyelination which are the typical features of multiple sclerosis in humans and both PPAR $\gamma$  and PPAR $\alpha$  agonists have been shown to exert therapeutic effects in the mouse model (Niino et al. 2001; Diab et al. 2002; Xu et al. 2007). Moreover, in a report of a single patient with severe multiple sclerosis, pioglitazone, a PPAR $\gamma$  activator, was found to substantially improve the patients condition (Pershadsingh et al. 2004). Those observations have now stimulated the call for large-scale clinical trials of PPAR-agonists for therapy of multiple sclerosis (Racke et al. 2006; Niino 2007). Since an inflammatory process in the brain is also observed in other severe conditions affecting the CNS such as Alzheimer's and Parkinson's disease, PPAR-agonists have also been tried (Landreth 2007; Dehmer et al. 2004) with promising results, thus justifying future trials of those drugs in various neurological disorders associated with neuroinflammation. It is of interest that not all effects of those receptor-agonists are mediated via the activation of PPAR, since some therapeutic effects are also observed with gemfibrozil, a PPAR $\alpha$  agonist, in PPAR $\alpha$ <sup>-/-</sup> mice with autoimmune encephalomyelitis (Dasgupta et al. 2007) suggesting receptor independent mechanisms of action. This is, however, mostly new territory where therapeutic benefits of those drugs remain to be discovered. The exact knowledge of the structure and function of peroxisomes and their heterogeneity

in the CNS, as reported recently (Ahlemeyer et al. 2007) is a prerequisite for such future studies.

### Mysterious players: identifying novel peroxisomal proteins

#### Peroxisomes in silico

The rapid advances in genome sequencing allow computational approaches (comparative genomics) to predict peroxisomal proteins. These in silico analyses take advantage of the conserved targeting sequences in peroxisomal proteins (i.e. PTS1, PTS2, see “Introduction” and Fig. 3). PTS domain-based comparisons across genomes from yeast to humans and plants have been performed, predicting large numbers of putative peroxisomal proteins (Emanuelsson et al. 2003; Kurochkin et al. 2005; Kamada et al. 2003; Reumann et al. 2004, database AraPeroX: <http://www.araperox.uni-goettingen.de/>; Hawkins et al. 2007). Some of the predictions have already been confirmed experimentally, others still await validation. There are, however, limitations, because many proteins of peroxisomes cannot currently be predicted from genome sequences. These include peripheral and integral membrane proteins, proteins that are imported in a piggy-back fashion, or matrix proteins with internal PTS1-like peptides. On the other hand, predicted PTSs can be masked by protein conformation, multimer formation, or posttranslational regulatory mechanisms.

#### Mysterious rise

Recently, computational approaches on the basis of proteomes and sequenced genomes of different organisms have been used to investigate the evolutionary origin of peroxisomes (Schluter et al. 2006; Gabaldon et al. 2006; Schluter et al. 2007, peroxisome database: <http://www.peroxisomedb.org/>). The peroxisomal proteins Pex3p, Pex19p, Pex10p, and Pex12p have been identified as markers for unequivocal in silico peroxisome detection (Schluter et al. 2006). The Apicomplexa phylum (containing for example, *Plasmodium falciparum*, *Cryptosporidium parvu*) has been detected as the first group of organisms devoid of peroxisomes in the presence of mitochondria. The absence of peroxisomes had so far only been documented in amitochondriate parasites, such as *Encephalitozoon cuniculi*, *Girardia lamblia*, or *Entamoeba histolytica*. Interestingly, a high degree of similarity among membrane proteins and between components of the matrix import machinery and the endoplasmic reticulum/proteasome degradation system has been observed (see “Mysterious formation: growth and division, de novo formation, or both?”)

(Schluter et al. 2006; Gabaldon et al. 2006). Most of the conserved peroxisome biogenesis and maintenance proteins are apparently eukaryotic innovations with no prokaryotic counterpart. This is consistent with findings that report an ER-dependent formation of peroxisomes (see “Mysterious formation: growth and division, de novo formation, or both?”) (Tabak et al. 2006), and indicates that peroxisomes do not have an endosymbiotic origin. In contrast, many peroxisomal matrix enzymes were found to have prokaryotic homologues. These might have been recruited individually from pools existing within the primitive eukaryote (e.g., from mitochondria), but an en bloc recruitment of this coherent collection of enzymes can also be envisaged (de Duve 2007). It is still an open (but interesting, and hotly debated) question, *when* the peroxisomes first appeared in the evolution of the eukaryotic cell, and if they might have been present before the adoption of mitochondria (Cavalier-Smith 1997).

#### Peroxisomes and mass spectrometry

The recent technical advances in two-dimensional gel electrophoresis (2D-GEP), liquid chromatography (LC), as well as mass spectrometry (MS) peptide identification and quantification laid the foundation for progress in organellar proteome studies, and have also been exploited to unravel the peroxisomal proteome. These studies depend not only on the availability of complete genome information or large EST collections of the species of interest for protein identification but also on the purity of the isolated organelle fraction. Due to the fragile nature of peroxisomes, the isolation of intact peroxisomes in high purity and sufficient quantity is difficult. However, sophisticated protocols have been developed for the isolation of mammalian peroxisomes, for fungi and yeast, as well as for plant peroxisomes (e.g., Völkl et al. 1996, 1999; Luers et al. 1998; Weber et al. 2004; Distel and Kragt 2006; Reumann et al. 2007).

One-dimensional (1D) and 2D-GEP of peroxisomes from rat liver followed by LC/tandem MS or MS resulted in the identification of more than 50 known constituents as well as novel peroxisomal proteins, among them a new isoform of Lon protease (one of the first peroxisomal proteases discovered) (Kikuchi et al. 2004), microsomal glutathione-S-transferase (Islinger et al. 2006), as well as the known microsomal proteins aldehyde dehydrogenase, cytochrome b5 and its corresponding reductase (Kikuchi et al. 2004; Islinger et al. 2006; Wiese et al. 2007). An early high throughput characterization of the yeast peroxisomal membrane proteome was performed by Schafer et al. (2001) using oleate-induced cultures. In order to differentiate between proteins that are contaminants of low abundance and those that have specifically enriched in the purified peroxisome fractions, efforts have been made to study relative

protein quantities. Aitchinson and coworkers introduced a relative quantitative MS-based proteomics approach (ICAT, isotope-coded affinity tagging) to determine the enrichment or depletion of proteins detected in two peroxisomal membrane fractions from *S. cerevisiae* that differed in their degree of purity, and revealed a role for the GTPase Rho1p in actin organization on the peroxisomal membrane (Marelli et al. 2004; Saleem et al. 2006). In another quantitative MS-based proteomics approach (iTRAQ, isobaric Tag for Relative and Absolute Quantitation) with peroxisome matrix and membrane subfractions from bezafibrate treated rats, Islinger et al. (2007) identified 134 individual proteins, including 15 new candidates, among them the new peroxisomal membrane proteins PMP52 (unknown function, related to PMP24), the iron-sulfur protein Mosc2 (MOCO sulphurase C-terminal domain containing 2 protein), and splice variants of the acyl-CoA binding protein ACBD5, as well as a trypsin-like serine protease (Tysnd1) of the peroxisomal matrix (Fig. 3). The mouse variant of this protease has been identified and characterized in detail (Kurochkin et al. 2007). Interestingly, Tysnd1 removes the leader peptide from PTS2 proteins and specifically processes PTS1 proteins involved in peroxisomal  $\beta$ -oxidation. Proteomic characterization of mouse kidney peroxisomes by tandem MS and protein correlation profiling has also led to the identification of 15 new peroxisomal candidates including Zadh2 (Zinc-binding alcohol dehydrogenase domain containing protein 2), Acad11 (acyl-CoA dehydrogenase family member 11), the aforementioned ACBD5, PMP52, Mosc2 (Wiese et al. 2007), as well as nudix hydrolase 19 (RP2) (Ofman et al. 2006). In addition, a quantitative proteomic comparison of mouse peroxisomes from liver and kidney has been performed (Mi et al. 2007b), as well as an age-related subproteomic analysis (Mi et al. 2007a). With the development of a purification method for leaf peroxisomes, Reumann et al. (2007) were able to perform a comprehensive proteome analysis of *Arabidopsis* leaf peroxisomes. They identified 42 novel proteins that had previously not been assigned to plant peroxisomes revealing novel targeting peptides, metabolic pathways, and defense mechanisms (Reumann et al. 2007). All these studies have contributed to a more complete picture of the peroxisome, and clearly demonstrate that we are not looking at an isolated structure, but rather on a dynamic compartment that is linked to (and might interact with) other subcellular structures (e.g. the ER, mitochondria, chloroplasts, the cytoskeleton, lipid droplets/bodies and other peroxisomes), and even shares proteins with them (in particular with the ER and mitochondria).

Although mass spectrometry can generally be more accurate than antibody-based detection methods, a validation of the proteins predicted to be peroxisomal is often required. The peroxisomal localization of some of the iden-

tified novel proteins has been experimentally confirmed by expression studies with tagged proteins, and by antibody-based approaches such as immunofluorescence microscopy and immunoblotting (e.g. Marelli et al. 2004; Islinger et al. 2006, 2007; Wiese et al. 2007).

In addition, large-scale localization studies have been performed in which the whole genome or a subset of the organisms' proteome was systematically tagged with GFP. The subcellular localizations of the GFP fusion proteins and their dynamics can then be addressed under live cell conditions, and several fusion proteins with peroxisomal localization have been identified by this approach (Cutler et al. 2000; Kumar et al. 2002; Huh et al. 2003; Natter et al. 2005).

Nevertheless, a lot of large-scale data are awaiting validation and contextualization. The great challenge for the future will be to provide a comprehensive and more complete picture of the relevant peroxisomal proteins, their abundance, functions, interactions and dynamic changes (Saleem et al. 2006). This can only be achieved by incorporating data from multiple sources, such as localization (see above) and interaction studies (e.g., two hybrid studies, analyses of immunisolated protein complexes), metabolite interactions, and microarray expression analyses.

## Concluding remarks

This review has highlighted recent novel discoveries in the peroxisome field. Ongoing studies on peroxisome biogenesis, formation and protein import have so far revealed unique features of peroxisomes, which have often been in disagreement with existing dogmas in cell biology. Contrary to mitochondria and ER, peroxisomal proteins can be imported in a completely folded or even oligomeric state, presumably via the formation of a transient membrane pore (Erdmann and Schliebs 2005). Recent findings also demonstrate that peroxisomes can be formed de novo from the ER or a subdomain of the ER (Hoepfner et al. 2005) in addition to growth and division of pre-existing peroxisomes (Yan et al. 2005; Schrader 2006; Fagarasanu et al. 2007). Furthermore, they appear to have an ER origin in evolutionary history (Gabaldon et al. 2006; Schluter et al. 2006). The physiological significance of the mechanism of de novo formation in comparison to the classical pathway of growth and division has again been challenged (Motley and Hettema 2007) and awaits further approval. In contrast to peroxisome division, de novo formation appears to be independent of dynamin-like proteins (Motley and Hettema 2007; Nagotu et al. 2007) and thus, the molecular machinery for de novo formation remains to be identified. Many new proteins affecting peroxisome growth, number, division, inheritance and turnover have been characterized

and much has been learned about their putative function, localization, and possible interaction with other proteins. The discovery that dynamin-related proteins like Vps1, DLP1/Drp1 and DLP/Drp-receptor proteins like Fis1p are required for peroxisomal fission has opened the field for the molecular characterization of the peroxisomal division machinery (Schrader and Fahimi 2006). Interestingly, peroxisomes and mitochondria share components of their fission machinery, underlining the tight cooperation and cross talk between both organelles (Schrader 2006; Schrader and Yoon 2007). It is still an open question, how the dual targeting of these components is mediated, and if organelle-specific components exist which distinctly regulate the assembly of the division machineries on both organelles. Furthermore, the mechanism of peroxisome constriction and of membrane phospholipid transfer during growth or de novo synthesis remains to be discovered. Another important question is, if and how peroxisomal morphology and dynamics influence peroxisomal functions as well as developmental and physiological processes. An improved understanding of the signalling pathways and the PPAR-mediated mechanism of peroxisome proliferation will further stimulate the investigation of the risks and benefits of peroxisome proliferators as therapeutic agents, for example in fatty liver disease or neuroinflammatory diseases.

There is firm evidence now for a semi-autonomous model of peroxisome formation, whereby the ER supplies existing peroxisomes with essential membrane proteins to allow growth and division. It will be a great challenge for the future to elucidate this trafficking pathway, which is supposed to involve ER-derived vesicular or pre-peroxisomal structures. Surprisingly, besides vesicular ER-to peroxisome transport, even a vesicular transport route between mitochondria and peroxisomes might exist (Neuspiel et al. 2008). It will therefore be of significant interest to unravel to what extent, why and how peroxisomes interact and exchange with other subcellular compartments, and whether they are themselves involved in the formation of vesicles. It is clear now that we are not looking at an isolated structure, but rather on a dynamic compartment that is linked to other subcellular structures, interacts with them and even shares proteins. There is no doubt that peroxisomes, which are still among the more mysterious subcellular compartments in eukaryotic cells, will reveal further surprises in the near future.

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