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## *Review*

# Peroxisome morphology in pathology

**Daniela Ribeiro[1], Inês Castro[1], H. Dariush Fahimi[2] and Michael Schrader[1]**

[1]Centre for Cell Biology and Deptartment of Biology, University of Aveiro, Aveiro, Portugal and [2]Department of Anatomy and Cell Biology, Division of Medical Cell Biology, University of Heidelberg, Heidelberg, Germany

**Summary.** Peroxisomes are remarkably dynamic and versatile organelles that are essential for human health and development. They respond to physiological changes in the cellular environment by adapting their morphology, number, enzyme content and metabolic functions accordingly. With the discovery of the first key peroxisomal morphology proteins, the investigation of peroxisomal shape, distribution and dynamics has become an exciting new field in cell biology and biomedical sciences because of its relation to organelle functionality and its impact on developmental and physiological processes. In this review, we summarize recent findings on peroxisome biology, dynamics and the modulation of peroxisome morphology, especially in mammals. Furthermore, we discuss the roles of peroxisome dynamics and morphology in cell pathology and present recent examples for alterations in peroxisome morphology under disease conditions. Besides defects in the peroxisomal morphology machinery, we also address peroxisome biogenesis disorders, alterations of peroxisome number during carcinogenesis and liver cirrhosis, and morphological alterations of peroxisomes during viral infection.

**Key words:** Peroxisomes, Organelle dynamics, Biogenesis, Dynamin, Pex11

## 1. Introduction

### *1.1. Classical and novel functions of peroxisomes*

Peroxisomes are ubiquitous, single-membrane bound organelles which are indispensable for human health and development (Islinger and Schrader, 2011). They belong to the basic equipment of the eukaryotic cell and are thus found in most animals, plants and fungi. Peroxisome functions are highly diverse depending on organism, cell type and developmental stage of the organism (Islinger et al., 2010). This led to their designation as a dynamic "multi-purpose organelle" with the remarkable capacity to respond to physiological changes in the cellular environment by adapting their number, morphology, enzyme content and metabolic functions accordingly.

Common functions of peroxisomes include the metabolism of hydrogen peroxide and lipids, especially the oxidation of fatty acids (van den Bosch et al., 1992; Wanders and Waterham, 2006a). The functional term "peroxisome" was introduced in 1965 by Christian De Duve (Nobel laureate in 1974). After isolation of the organelle from rat liver, he and his colleagues co-localized several $H_2O_2$-producing oxidases, as well as catalase, a $H_2O_2$–degrading enzyme, in the organelle matrix. In addition, several specialized functions have been evolved with plants, yeasts and protozoa generally possessing a broader spectrum of peroxisomal activities than vertebrates. These include penicillin biosynthesis and degradation of methanol in fungi, glycolysis in trypanosomes, photorespiration and glyoxylate cycle in plants, and plasmalogen biosynthesis in mammals. Etherlipids such as plasmalogens are important constituents of the neuronal myelin sheaths in the brain, and loss of peroxisomal functions is often associated with neurodegenerative processes (Faust et al., 2005; Hulshagen et al., 2008). Additionally, peroxisomes in mammals are involved in the synthesis of bile acids, and docosahexanoic acid (a modulator of neuronal function), fatty acid elongation, α- and ß-oxidation of certain fatty acids (e.g. phytanic acid and very long chain fatty acids, respectively), metabolism of amino acids, catabolism of purines, polyamines, and mediators of inflammation

*Offprint requests to:* Michael Schrader, Centre for Cell Biology & Dept. of Biology, University of Aveiro, Campus Universitário Santiago, 3810-193 Aveiro, Portugal. e-mail: mschrader@ua.pt

**Abbreviations:** ER, endoplasmic reticulum; PBD, peroxisome biogenesis disorder; PEX, peroxin; PMP, peroxisomal membrane protein; PPAR, peroxisome proliferator activated receptor; PTS, peroxisomal targeting signal; ROS, reactive oxygen species

such as prostaglandins and eicosanoids (van den Bosch et al., 1992; Wanders and Waterham, 2006a; Islinger et al., 2010). The evolutionary loss of peroxisomal urate oxidase makes humans susceptible to gout, a disease completely absent in mammals that possess a functional urate oxidase (Hayashi et al., 2000).

To fulfil the multitude of diverse functions, peroxisomes have to interact, cooperate and cross-talk with other subcellular compartments, for example with mitochondria (see 2.1.1.), the endoplasmic reticulum, lipid droplets or chloroplasts. In animals, peroxisomes and mitochondria possess their own set of ß-oxidation enzymes, but cooperate in the degradation of fatty acids to maintain lipid homeostasis (Wanders, 2004b). Interestingly, in yeasts and plants the ß-oxidation of fatty acids solely depends on peroxisomes (Poirier et al., 2006). Furthermore, peroxisomes and mitochondria were recently shown to cooperate in the synthesis of biotin in fungi (Tanabe et al., 2011). Such a degree of metabolic interactions and cross-talk renders peroxisomes and mitochondria dependent upon each other for their function and requires a coordinated biogenesis and turnover (Schrader and Yoon; 2007; Camões et al., 2009).

Besides the classical peroxisomal functions mentioned above, several additional peroxisome functions, including non-metabolic ones have been recently discovered. Bioinformatic approaches to predict peroxisomal proteins and proteomics studies combined with validation experiments have led to the discovery of novel proteins and pathways in peroxisomes, especially in plants (Reumann et al., 2009), where peroxisomes were shown to synthesize phytohormones which regulate important physiological processes such as growth and senescence. Root peroxisomes of tropical legumes were demonstrated to synthesize allantoin as a carrier for nitrogen transport, and fungal peroxisomes were recently associated with the synthesis of biotin (Lamberto et al., 2010; Tanabe et al., 2011).

Besides catalase, peroxisomes harbour several other enzymes involved in the production and scavenging of reactive oxygen species (ROS) (e.g. superoxide dismutases, peroxiredoxins, glutathione peroxidase, epoxide hydroxylase) (del Río et al., 2006; Schrader and Fahimi, 2006a; Bonekamp et al., 2009). Thus, their contribution to ROS metabolism, oxidative stress, ageing, neurodegeneration and carcinogenesis has received great attention (Cimini et al., 2009; Kou et al., 2011; Titorenko and Terlecky, 2011). Another important aspect is the function of their metabolites in cellular signalling and the role of peroxisomes as signalling platforms during development, differentiation and host defence (Masters, 1996; Titorenko and Rachubinski, 2004). It has been shown that peroxisomes play a role in ROS and reactive nitrogen species signalling (Del Río, 2011). Furthermore, a new biological function for mammalian peroxisomes in antiviral innate immunity and antiviral signalling was discovered (Dixit et al., 2010) (see 2.4.).

### *1.2. Dynamic peroxisome formation*

In recent years it has become evident that peroxisomes can form by growth and division of pre-existing organelles, whereby the ER provides membrane lipids for growth and ongoing fission (Schrader and Fahimi, 2006b; Hettema and Motley, 2009; Nagotu et al., 2010). Interestingly, peroxisomes can also arise from the ER via a maturation process. This *de novo* formation is observed in mutant cells devoid of peroxisomes after reintroduction of the deficient gene (encoding the membrane biogenesis/import factors Pex19p, Pex3p, or Pex16p) or in yeast mutants lacking peroxisomes due to a segregation defect (Hoepfner et al., 2005; Nagotu et al., 2008, 2010). Findings in yeast indicate that in wild type cells peroxisomes multiply by growth and division and do not form *de novo* (Motley and Hettema, 2007; Nagotu et al., 2008). However, simultaneous formation by both processes has also been suggested (Kim et al., 2006; van der Zand et al., 2010; Saraya et al., 2011). Interestingly, *de novo* formation of peroxisomes from mitochondria was very recently induced upon mitochondrial targeting of Pex3p, a peroxin involved in peroxisomal membrane biogenesis and a key player in *de novo* formation from the ER (Rucktäschel et al., 2010). This indicates that natural or artificial targeting of Pex3p to any endomembrane may initiate peroxisome formation.

Growth and division of peroxisomes in mammals follows a multistep maturation pathway involving a well defined sequence of morphological alterations including elongation, membrane constriction, and final fission (Fig. 1) (Schrader et al., 1996; Schrader and Fahimi, 2006b). Division of peroxisomes is preceded by elongation of the organelle membrane, which requires the activity of the membrane-deforming, conserved peroxisomal membrane protein (PMP) Pex11p (Schrader et al., 1998b; Thoms and Erdmann, 2005). In recent years, great progress has been made in the identification of key factors involved in the fission of peroxisomes (Fig. 1) (see 2.1.). Final fission depends on dynamin-related-proteins, large GTPases with mechanochemical properties, and associated adaptor proteins (e.g. DLP1/Drp1 and its membrane adaptors Fis1 and Mff in mammals) (see 2.1.). Interestingly, peroxisomes and mitochondria have been shown to share key components of their division machinery, which appears to be a common strategy used by mammals, fungi and plants (Schrader, 2006, 2009). Notably, peroxisome formation from either the ER or by growth and division appears to follow a maturation process involving the recruitment of new membrane and matrix proteins (Delille et al., 2010, 2011). We recently demonstrated that Pex11pß-mediated growth (elongation) and division of mammalian peroxisomes follows a multistep maturation pathway, which is initiated by the formation of an early peroxisomal membrane compartment from a pre-existing peroxisome and its stepwise conversion into a mature, metabolically active peroxisome compartment (Fig. 1).

Maturation is achieved by selective and stepwise import of certain PMPs, membrane lipids and matrix proteins. Our observations support the view that peroxisome division is an asymmetric process (Huybrechts et al., 2009), which is far more complex than simple (symmetric) division of a pre-existing organelle (Delille et al., 2010, 2011).

It is well established that peroxisomal matrix proteins are all synthesized on free ribosomes in the cytoplasm and are post-translationally imported into the organelle. Two peroxisomal targeting signals (PTS1 and PTS2) have been defined, which are recognized by soluble receptors (Pex5p and Pex7p, respectively) that interact with a receptor docking site at the peroxisomal membrane. Peroxisomal matrix protein import has been addressed in several excellent review articles (Ma et al., 2011; Rucktäschel et al., 2011). It involves a unique protein translocation machinery that can handle folded, co-factor bound and oligomeric proteins. The Pex5p receptor is supposed to insert into the peroxisomal membrane and to form a large transient pore for cargo translocation (Meinecke et al., 2010). Pex5p is then recycled depending on ubiquitination events. The import of PMPs requires a different, less characterised protein machinery which involves the cytosolic receptor/chaperone Pex19p. The peroxisomal membrane proteins Pex3p and Pex16p interact with the Pex19p-PMP complex to mediate membrane insertion. Their loss of function leads to peroxisome absence, for example in Zellweger patients (Pex16 and Pex19) and in neonatal adrenoleukodystrophy patients (Pex3) (Steinberg et al., 2006). It is currently controversial whether all PMPs (like the peroxisomal matrix proteins) are translated in the cytoplasm or if PMPs are first inserted into the ER from where they bud in specialized vesicles which are finally delivered to peroxisomes (van der Zand et al., 2010; Agrawal et al., 2011; Lam et al., 2011; Nuttall et al., 2011).

### *1.3. Modulation of peroxisome number and morphology*

Peroxisomes are remarkably dynamic organelles, which react to physiological changes in their cellular environment and adapt their number, morphology, enzyme content and metabolic functions accordingly. Pharmacological studies with hypolipidemic drugs and plasticizers (so called "peroxisome proliferators" which include divergent drugs, industrial chemicals such as lubricants, plasticizers and agrochemicals) revealed 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). Those compounds are supposed to mimic signals, which activate intracellular signalling cascades and lead to the activation of transcription factors (e.g. PPAR-α, PPAR-γ) (Fig. 2). Such a "peroxisome proliferation" is often accompanied by an increased synthesis of peroxisomal enzymes (e.g. involved in fatty acid ß-oxidation). In addition, peroxisomes with elongated forms, as well as interconnections suggestive of peroxisome division (see 2.1.; Fig. 1), were detected in studies with bezafibrate (Fahimi et al., 1993) and other hypolipidemic drugs (Lindauer et al., 1994; Fahimi et al., 1996). Interestingly, elongated and constricted peroxisomes were more prominent in periportal hepatocytes, in contrast to more spherical ones in pericentral hepatocytes indicative of zonal heterogeneity in response to hypolipidemic drugs (Fahimi et al., 1996). Prolonged treatment with peroxisome proliferators has a carcinogenic effect in

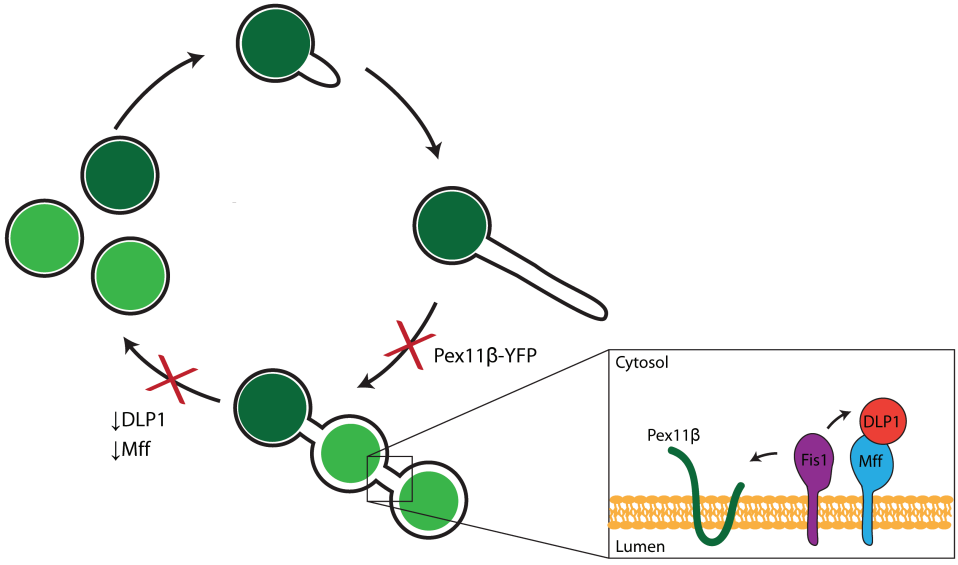


**Fig. 1.** Model of peroxisome proliferation by growth and division. Formation of new peroxisomes starts with the development of a tubular extension from a mature peroxisome. Upon growth, this extension segments and constricts and new proteins are imported to the forming peroxisomes (this step can be inhibited by the expression of a Pex11ß-YFPm fusion protein) (Delille et al., 2010). Final division is performed by DLP1, a dynamin-like-protein which is recruited to the membrane by Mff and Fis1, two tail-anchored membrane proteins. Fis1 has been shown to interact with the membrane elongation factor Pex11ß. Loss of DLP1 or Mff results in the formation and accumulation of constricted peroxisomal tubules ("beads-on-a-string"-like morphology) (see Fig. 3).

rodents leading to liver tumors (Reddy et al., 1980, 1982; Moody et al., 1991) (see 2.3.). Peroxisome proliferation can also be induced e.g. by fatty acids, cold adaptation or exposure to environmental pollutants (Schrader and Fahimi, 2008) (Fig. 2). In yeast, peroxisome proliferation (e.g. upon growth on oleic acid or methanol as the sole carbon source) has been exploited to identify peroxisome biogenesis (PEX) mutants (Purdue and Lazarow, 2001). In plants, peroxisome proliferation can be induced by stress conditions (e.g. wounding, pathogen attack, drought, osmotic stress, excess light) that generate $H_2O_2$ as a signalling molecule (Lopez-Huertas et al., 2000; Desikan et al., 2001; del Río et al., 2002). A rapid formation of peroxisomal tubular extensions in plant cells after exposure to $H_2O_2$ has been reported (Sinclair et al., 2009). Formation of these extensions was later followed by peroxisome elongation and division, suggesting the existence of a rapid, transcription-independent pathway and a transcription-dependent pathway in peroxisome response to $H_2O_2$/oxidative stress.

The classical peroxisome proliferators in mammals (e.g. fatty acids, hypolipidemic fibrates) bind to the nuclear transcription factor PPAR-α, which belongs to the superfamily of steroid/thyroid/retinoid receptors (originally designated as peroxisome proliferator activated receptor) (Issemann and Green, 1990; Rakhshandehroo et al., 2010) (Fig. 2). The PPAR subtypes α, ß and γ were identified in different species. The PPARs form heterodimers with retinoid X receptors (RXRs) and induce the transcription of peroxisomal genes involved in ß-oxidation and proliferation (e.g. PEX11) by binding to specific DNA-sequences known as peroxisome proliferator response elements (PPREs) (Fig. 2). PPREs have been reported for all peroxisomal ß-oxidation enzymes but also for several microsomal cytochrome P-450 subtypes and for apolipoproteins type I and II (Schoonjans et al., 1996). Activation of PPAR-α affects numerous metabolic pathways (Reddy and Hashimoto, 2001; Rakhshandehroo et al., 2010). As metabolic activities have also been shown to affect the number of peroxisomes (Chang et al., 1999; Funato et al., 2006), PPAR-α dependent peroxisome proliferation might be mediated through some kind of metabolic control. Additional PPAR-α independent mechanisms have been suggested. Peroxisome proliferation during cold adaptation in brown adipose tissue involves PPAR-γ and its co-activator PGC-1α (Bagattin et al., 2010).

Another important model system of peroxisome proliferation in mammals is the regenerating rat liver after partial hepatectomy (Yamamoto and Fahimi, 1987a). Regenerating rat liver peroxisomes are pleomorphic, often exhibiting tail-like extensions, elongated forms and constricted, interconnected structures indicative of peroxisome division (see 2.2.; Fig. 1). Such peroxisomes were visualized by catalase cytochemistry (Fahimi, 1969), and their inter-relationships were analysed by computer-assisted serial section reconstruction. The complex peroxisomal structures were frequently observed 24-48 hours after partial hepatectomy, which is the phase of rapid hepatic growth, and declined in frequency after 96 hours (Yamamoto and Fahimi, 1987b). Interestingly, similar peroxisome morphologies were observed in cell cultures of mammalian cells, for example in human hepatoblastoma HepG2 cells (Schrader et al., 1996). The frequency of cells exhibiting elongated and constricted peroxisomes was found to be dependent on cell density and serum components/growth factors and could be modulated accordingly (Schrader et al., 1996, 1999). Peroxisome elongation was strongly stimulated under conditions of cellular growth, for example by low cell

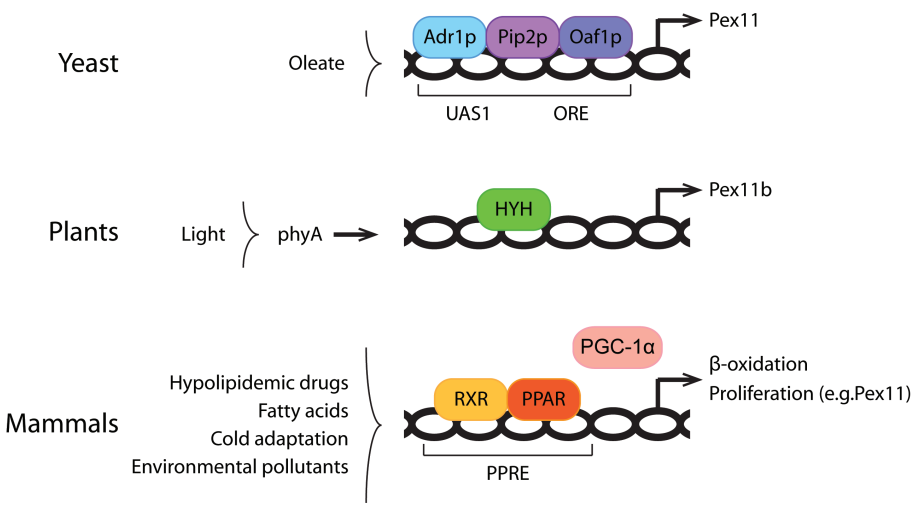


**Fig. 2.** Activation of peroxisome proliferation in yeast, plants and mammals. In most organisms, peroxisome proliferation is preceded by the induction of genes associated with fatty acid ß-oxidation and membrane elongation (e.g. Pex11). Activation of these pathways depends on several environmental and developmental conditions. In yeast, growth in the presence of oleic acid induces the dimerization of the transcription factors Oaf1p and Pip2p that, together with Adr1p, bind the oleate response element (ORE) and upstream activation sequence 1 (UAS1). In plants, light induces the expression of peroxisomal genes by the action of phyA and the binding of the transcription factor HYH to the PEX11b promoter. In mammals, peroxisome proliferator-activated receptor alpha (PPAR-α) and retinoid X receptor (RXR) coordinately bind to the PPAR response element (PPRE) to upregulate gene expression. Recently, other mechanisms independent of PPAR-α have also been described (e.g. PGC-1α dependent) (Bagattin et al., 2010).

density and after addition of polyunsaturated fatty acids (e.g. arachidonic acid) or certain growth factors. In addition, peroxisome elongation was experimentally induced by depolymerization of microtubules (Schrader et al., 1996), exposure of cells to UV irradiation and ROS (Schrader et al., 1998a), Pex11p overexpression (Schrader et al., 1998b; Lingard and Trelease, 2006) (see 2.1.2.), depletion of Sec16B (Yonekawa et al., 2011), or by inhibition of DLP1/Drp1, Fis1 or Mff function (Koch et al., 2003; Tanaka et al., 2006) (see 2.1.1.). Whereas the latter manipulations act directly on the peroxisomal membrane/the growth and division machinery, the other conditions involve intracellular signalling to induce peroxisome elongation (Schrader et al., 1998a). However, most of the signalling cascades and the gene responses leading to peroxisome elongation/proliferation are still largely unknown. The expression and/or activation of Pex11p appears to be a common, initial event in yeasts, plants and mammals during peroxisome proliferation (Fig. 2). Elongated peroxisomes are formed upon expression of Pex11p in all organisms studied (Schrader et al., 1998b; Thoms and Erdmann, 2005). Pex11p functions as a membrane-shaping protein, which directly deforms the peroxisomal membrane prior to fission (Koch et al., 2004; Delille et al., 2010; Koch et al., 2010). This activity may result from amphiphatic properties found in the N-terminal part of many Pex11 proteins (Opaliński et al., 2011). The signalling mechanisms acting upstream of Pex11p differ in yeasts, plants and mammals (Fig. 2) (Kaur and Hu, 2009).

Obviously, one important function of elongated peroxisomes is to multiply/proliferate peroxisomes by growth and division according to cellular needs. However, complex peroxisomal structures such as elongated tubules or tubulo-reticular networks may also contribute to and facilitate other peroxisomal processes (e.g. in metabolism, metabolite transport, membrane signalling, stress protection) although information on their exact functions is scarce. Morphologically distinct types of peroxisomes have been discovered in different organs of mammalian organisms and cell lines (Hicks and Fahimi, 1977; Gorgas, 1987; Yamamoto and Fahimi, 1987a; Roels et al., 1991; Fahimi et al., 1993; Schrader et al., 1994, 1996, 2000; Litwin and Bilinska, 1995). Tubular peroxisomes with interconnections forming a "peroxisomal reticulum" have been reported in mouse liver (Gorgas, 1985), as well as in lipid synthesizing epithelia such as sebaceous glands of mammals and uropigial glands of birds (Gorgas, 1987). Tubulo-reticular peroxisomes have been observed to be extremely dynamic, with constant formation of tubular extensions interconnecting or detaching (Schrader et al., 2000; Koch et al., 2004), and are frequently associated with lipid droplets (Schrader, 2001). The extensive and complex structure of the "peroxisomal reticulum" in lipid synthesizing epithelia is consistent with the rapid formation and turnover of the peroxisomal compartment, which is involved in the biosynthesis of secretory lipids (Kollatakudy et al., 1987). More complex peroxisomal structures such as tubules or reticular networks may facilitate the metabolic functions of peroxisomes by generating a uniform biochemical distribution of proteins, by increasing the surface to volume ratio and the exchange of metabolites, thus being more efficient in intracellular areas of greatest metabolic need. In line with this, overproduction of Pc-Pex11p in the fungus *P. chrysogenum* induced massive proliferation of tubular peroxisomes and increased penicillin production without affecting the levels of the enzymes of the penicillin biosynthetic pathway (Kiel et al., 2005).

An elongated morphology might also protect peroxisomes from autophagosomal destruction under certain conditions (as has been recently described for mitochondria during nutrient starvation) (Gomes et al., 2011; Rambold et al., 2011), whereas a shift to fission could render them prone to degradation or trigger pexophagy. Interestingly, high levels of Fis1, which distributes to mitochondria and peroxisomes, have been demonstrated to induce apoptosis (James et al., 2003) and to trigger autophagy (Gomes and Scorrano, 2008). Peroxisomal shape will also affect peroxisomal movement and distribution, e.g. in neurons or during inheritance (Nagotu et al., 2008). Kinesin motors and dynein/dynactin control the movement of peroxisomes along microtubules in mammals and thus their proper intracellular distribution (Schrader et al., 2003). Peroxisome division may generate smaller peroxisomes which can be readily moved by the motor proteins, whereas more complex peroxisomal structures are likely to be more stationary.

## 2. Morphological alterations of peroxisomes in human disease

### *2.1. Defects in peroxisome morphology proteins*

The investigation of peroxisomal and mitochondrial cross-talk/interconnection, morphology and dynamics has become an exciting new field in cell biology and biomedical sciences because of its relation to organelle functionality and its impact on developmental and physiological processes. There is growing evidence now that mitochondrial morphology and dynamics are crucial for cell physiology. Changes in mitochondrial shape influence important cellular functions, such as calcium signalling, generation of ROS, apoptosis, autophagy and cell death, neuronal plasticity, muscle atrophy, and even lifespan (for recent reviews see Campello and Scorrano, 2010; Westermann, 2010; Gomes et al., 2011). Mitochondrial morphology is determined by balanced fusion and fission events, which are controlled by "mitochondria-shaping" proteins (Palmer et al., 2011b). The key components for mitochondrial fusion (e.g. the mitofusion proteins Mfn1 and Mfn2 or OPA1) and fission (e.g. DLP1/Drp1) are high-molecular weight GTPases (Praefcke and McMahon, 2004) (reviewed in Okamoto and Shaw, 2005; Hoppins et al., 2007)

Information on the importance of peroxisomal

morphology, intracellular distribution and movement for cellular physiology is still limited, but the field is young and many peroxisome morphology proteins have just recently been discovered and functionally characterized (Thoms and Erdmann, 2005; Schrader, 2006; Delille et al., 2009; Opaliński et al., 2011). Similar to mitochondria, peroxisomal dysfunctions are often associated with neurological and developmental defects and have been linked to pathological conditions associated with oxidative stress and to ageing (Cimini et al., 2009; Kou et al., 2011; Titorenko and Terlecky, 2011).

In contrast to mitochondrial dynamics, which are based on organelle fusion and fission, only peroxisomal fission events have so far been reported. There is growing evidence that mature peroxisomes in yeast and mammals do not fuse in a mechanism similar to mitochondrial fusion (Motley and Hettema, 2007; Huybrechts et al., 2009; Bonekamp et al., 2010a). Mitochondrial fusion proteins such as the dynamin-related GTPases Mfn1 and Mfn2, or OPA1 were not found to localize to mammalian peroxisomes (Bonekamp et al., 2010a). Interestingly, live cell imaging revealed that peroxisomes were engaged in transient and long term contacts, but without exchanging matrix or membrane markers (Bonekamp et al., 2010a).

#### 2.1.1. DLP1 deficiency

The dynamin-like large GTPase DLP1/Drp1 as well as Fis1 (Fission factor 1) and Mff (Mitochondrial fission factor), two potential membrane adaptors for DLP1, represent key components of the peroxisomal fission machinery in mammals (Koch et al., 2003, 2004, 2005; Li and Gould, 2003; Tanaka et al., 2006; Kobayashi et al., 2007; Gandre-Babbe and van der Bliek, 2008; Otera et al., 2010) (Fig. 1). In contrast to the key fusion proteins, these components are shared with the mitochondrial division machinery (Schrader and Yoon, 2007; Camões et al., 2009; Delille et al., 2009). DLP1 is a conserved dynamin GTPase superfamily protein which is supposed to assemble into higher ordered ring-like structures in a GTP-dependent manner that wrap around membrane tubules to sever the membrane in a GTP hydrolysis-dependent process (Praefcke and McMahon, 2004). DLP1 activity is highly regulated through a number of post-translational modifications including phosphorylation, S-nitrosylation, ubiquitination and sumoylation (Santel and Frank, 2008; Chang et al., 2010). Fis1 and Mff are both C-tail anchored membrane proteins. Fis1 contains an N-terminal tetratricopeptide repeat (TPR) motif, whereas Mff exposes its N-terminal part with a central coiled-coil motif into the cytosol (Yoon et al., 2003; Gandre-Babbe and van der Bliek, 2008). The role of Fis1 as the main adaptor for DLP1 in mammals and its contribution to organelle fission has recently been questioned and a major function for Mff has been proposed (Otera et al., 2010). The physiological role of Fis1 is currently under discussion, but it might contribute to the regulation of organelle division (Dikov and Reichert, 2011; Otera and Mihara, 2011; Palmer et al., 2011a; Zhao et al., 2011). Fis1 is targeted to peroxisomes in a Pex19p-dependent manner (Delille and Schrader, 2008) and proper targeting requires the short C-terminal tail (Koch et al., 2005). On peroxisomes, Fis1 is supposed to interact with Pex11ß (Kobayashi et al., 2007), and is found to co-localize with Pex11ß in tubular membrane domains of peroxisomes which are formed prior to division (Delille et al., 2010). Physical interactions among all five At-Pex11p isoforms and Fis1b have also been reported in *Arabidopsis* (Lingard et al., 2008).

Functional loss of DLP1 (or its adaptor proteins Fis1 and Mff) by siRNA-mediated silencing or the expression of dominant-negative mutants has been shown to inhibit peroxisomal (and mitochondrial) fission, resulting in an elongated organelle morphology (Koch et al., 2003, 2004, 2005; Li and Gould, 2003; Kobayashi et al., 2007; Gandre-Babbe and van der Bliek, 2008; Otera et al., 2010). Interestingly, the elongated peroxisomes observed after silencing of DLP1 are still capable of constricting their membranes and thus have a segmented, "beads on a string"-like appearance (Koch et al., 2004) (Figs. 1, 3). This indicates that DLP1 is not required for peroxisome constriction, but for final membrane scission. The molecular components mediating peroxisomal constriction are still unknown.

Based on the description of an elongated, constricted peroxisome morphology after silencing of DLP1 in mammalian cells (Koch et al., 2003, 2004), a novel lethal disorder with defects in both peroxisomal and mitochondrial division based on a mutation in DLP1 was identified (Waterham et al., 2007). This novel DLP1 deficiency appears to be the first member of a new group of combined peroxisomal-mitochondrial disorders. The patient, who died only few weeks after birth, showed microcephaly, abnormal brain development, optic atrophy and hypoplasia. Several of the abnormalities were broadly similar to known disorders related to mitochondrial dynamics (e.g. Charcot-Marie-Tooth neuropathy, autosomal dominant optic atrophy), but the clinical course was more severe. Furthermore, elevated plasma levels of lactate and very long chain fatty acids were detected, indicating defects in both mitochondrial and peroxisomal functions (mitochondrial respiration and peroxisomal ß-oxidation, respectively). Skin fibroblasts from the patient displayed elongated, constricted peroxisomes (and elongated mitochondria) similar to the ones described after loss of DLP1 function. Genetic analysis revealed a heterozygous, dominant-negative missense mutation (A395D) in the middle domain of DLP1 (Waterham et al., 2007). It has recently been demonstrated that this mutation inhibits oligomerization of DLP1 (Chang et al., 2010). So far, only this middle domain mutation has been reported in a patient. However, other middle domain mutations (e.g. C452F; G363D, which may interfere with GTP hydrolysis) (Tanaka et al., 2006; Ashrafian et al., 2010)

or mutations in the GTPase domain (e.g. K38A, which inhibits GTP hydrolysis, but does not affect GTP binding) are known to inhibit DLP1 function and to result in an elongated organelle morphology (Koch et al., 2003; Li and Gould, 2003). Meanwhile, DLP1 knockout mice have been generated (Ishihara et al., 2009; Wakabayashi et al., 2009) which display developmental abnormalities that result in embryonic lethality. Peroxisomes and mitochondria show an elongated and constricted morphology after knock-out of DLP1, thus confirming siRNA and mutational studies in cell culture. DLP1 appears to be essential for synapse formation and brain development in mice, e.g. by producing mitochondria, whose size is compatible with their movement and proper distribution within neurons. Interestingly, loss of DLP1 has no effect on mitochondrial ATP production. However, a C452F mutation in the DLP1 middle domain has been described to result in dilated cardiomyopathy in mice (Ashrafian et al., 2010).

It is presently unclear to what extent defects in mitochondrial or in peroxisomal function contribute to the clinical phenotype of the patient and to the pathological alterations observed in the knock-out models. Furthermore, DLP1 may fulfil additional functions in other subcellular locations. Recently, DLP1 has been localized to the Golgi complex in distinct cell types and a role as a novel component of the apical sorting machinery at the trans-Golgi network has been proposed (Bonekamp et al., 2010b).

Although a patient with mutations in Mff has not been reported yet, loss of Mff function by siRNA mediated silencing results in elongated peroxisomes (and mitochondria) similar to silencing of DLP1 (Gandre-Babbe and van der Bliek, 2008; Otera et al., 2010). We also observed that the elongated peroxisomes formed after silencing of Mff have a constricted morphology, as reported for silencing of DLP1 (Koch et al., 2003, 2004) (our unpublished results) (Fig. 3). This indicates that Mff (like DLP1) is likely not involved in the constriction of peroxisomes prior to fission. Furthermore, an elongated and constricted peroxisome morphology in patient fibroblasts may be the result of mutations in either DLP1 or Mff. Thus, both genes should be analyzed for potential mutations.

These findings also indicate that the analysis of peroxisomal (and mitochondrial) morphology in patient cells is a valuable diagnostic tool for the determination of disorders based on defects in peroxisomal (mitochondrial) division.

### 2.1.2. Pex11 deficiency

Pex11 proteins are unique components of the peroxisomal division and proliferation machinery, and are supposed to play a major role in the regulation of peroxisomal growth in size and number in lower and higher eukaryotes. A loss of Pex11 is usually accompanied by a reduced peroxisome number/abundance and the formation of enlarged peroxisomes, whereas overexpression promotes peroxisome elongation and proliferation (Thoms and Erdmann, 2005; Yan et al., 2005; Schrader and Fahimi, 2006b; Delille et al., 2010; Koch et al., 2010; Opaliński et al., 2011). In *S. cerevisiae*, ScPex11p action has recently been shown to be regulated by phosphorylation (Knoblach and Rachubinski, 2010). In mammals three Pex11 proteins (α, ß, and γ) exist, which are supposed to be integral membrane proteins with their N- and C-termini exposed to the cytosol. All Pex11 proteins interact with themselves, and are likely to form homo-oligomers or homo-dimers (Thoms and Erdmann, 2005; Koch et al., 2010). Pex11pα, but not Pex11pß or Pex11pγ, is induced by peroxisome-proliferating agents.

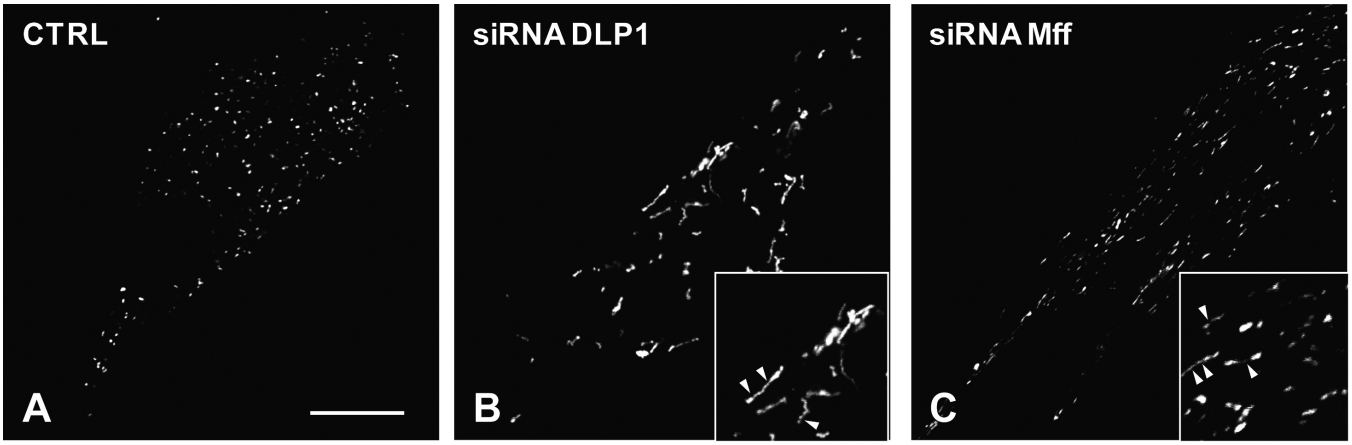


**Fig. 3.** Silencing of DLP1 or Mff leads to an elongated, constricted peroxisome morphology. Human fibroblasts were transfected with DLP1 **(B)** or Mff **(C)** siRNA duplexes (siRNA) and processed for immunofluorescence with anti-Pex14 antibodies after 48 hours. **A.** Control cells (CTRL) (transfected with luciferase siRNA duplexes). Note the segmented appearance of the elongated peroxisomes (see arrowheads in higher magnification inserts). Bar: 10 *μ*m.

Expression of Pex11pα varies between different tissues (being highest in kidney, liver and testis) (Abe et al., 1998; Passreiter et al., 1998; Schrader et al., 1998b; Li et al., 2002a; Tanaka et al., 2003), whereas Pex11pß is expressed in most tissues (Abe and Fujiki, 1998, Schrader et al., 1998b). Pex11pß is not directly involved in final fission of peroxisomes, and most likely functions upstream of DLP1 by promoting peroxisome elongation (Koch et al., 2003, 2004; Kobayashi et al., 2007) (Fig. 1). Pex11α and Pex11ß knock-out mice have been generated (Li et al., 2002a,b). The Pex11α knock-out mouse is viable, with no obvious effect on peroxisome number or metabolism, and morphologically indistinguishable from wild type mice (Li et al., 2002a). In contrast, knock-out of Pex11ß causes neonatal lethality and is accompanied by several defects reminiscent of Zellweger syndrome, a peroxisome biogenesis disorder (see 2.2.) (e.g. developmental delay, hypotonia, neuronal migration defects and neuronal apoptosis) (Li et al., 2002b). However, protein import into peroxisomes and peroxisomal metabolism is only slightly affected. Peroxisome abundance was reported to be reduced in cultured mouse Pex11ß$^{-/-}$ fibroblasts. Pex11α/Pex11ß double knock-out mice also show neonatal lethality and severe neurological defects, but do not exhibit a more severe phenotype than knock-out of Pex11ß alone. The mice still contain peroxisomes and are only mildly affected in peroxisomal metabolism (Li et al., 2002a). A recent comparative analysis of primary neuronal cultures and brain samples from Pex11ß$^{-/-}$, Pex11ß$^{+/-}$, and wild-type mice revealed a higher degree of cell death in heterozygous than in wild type mice (Ahlemeyer et al., 2011). Homozygous knock-out mice exhibited the highest degree of cell death, which correlated with a decrease in peroxisome number in the Pex11ß$^{-/-}$, but not in the Pex11ß$^{+/-}$ mice. Heterozygotes also showed delayed neuronal differentiation and increased levels of oxidative stress, which were, however, more pronounced in homozygotes. Thus, deletion of a single allele of the Pex11ß gene is sufficient to cause neurological alterations in mice (Ahlemeyer et al., 2011). Interestingly, an increase in the mRNA level of Pex11γ was detected in neuronal cultures of Pex11ß$^{-/-}$ mice, but not in those from Pex11ß$^{+/-}$ mice, whereas the mRNA levels of Fis1 and DLP1 remained unaffected.

Careful analysis of peroxisome morphology in patient cells may help to identify patients with defects in PEX11 or other peroxisome morphology proteins. As those patients may display no (or only mild) biochemical alterations of peroxisome metabolism, a connection to peroxisome dysfunction can be overlooked or misinterpreted, e.g. as a mitochondrial dysfunction.

### 2.2. Peroxisome biogenesis disorders and single enzyme deficiencies

Peroxisome function is crucial for cellular homeostasis and the vitality and development of the organism. This is best illustrated by the occurrence of several inherited peroxisomal disorders in humans that are often lethal (Weller et al., 2003; Steinberg et al., 2006; Wanders and Waterham, 2006b). Peroxisomal disorders are grouped into the more frequent single enzyme deficiencies (for example, defects in fatty acid transporters as in X-linked adrenoleukodystrophy) and the more severe peroxisomal biogenesis disorders (PBD) (e.g. Zellweger syndrome). A defect in a peroxisomal gene can result in a single enzyme deficiency which might affect one specific peroxisomal function or metabolic pathway (e.g. fatty acid ß-oxidation) (see below). In the case of PBDs, the affected protein is a peroxin, which is involved in the biogenesis and maintenance of peroxisomes (see 1.2.). Therefore, several or all peroxisomal functions can be impaired, and peroxisomes are either completely absent (due to a defect in their formation/membrane biogenesis based on mutations in PEX3, PEX19 or PEX16) (see 1.2.) or "empty" membrane compartments, so called "ghosts", which cannot fully develop and mature (due to a defect in the import machinery for peroxisomal enzymes, e.g. based on mutations in the import receptor PEX5). Targeting and insertion of peroxisomal membrane proteins to those membrane remnants appears to be functional, and overexpression of Pex11p has been reported to induce proliferation of peroxisomal ghosts in PEX5-deficient cells (Li and Gould, 2002). Furthermore, peroxisomal remnants appear to be motile in mammalian cells (Bharti et al., 2011). However, the peroxisomal matrix proteins remain in the cytosol, where they cannot function or are degraded. A loss of peroxisomal metabolic functions is generally accompanied by the accumulation of toxic substrates (e.g. very long chain fatty acids, phytanic acid), which can only be handled by peroxisomes, and a shortage of peroxisomal products (e.g. myelin sheath lipids). Symptoms include neonatal hypotonia, craniofacial dysmorphy, myelination and neuronal migration defects, seizures, hepatomegaly, liver cirrhosis, and renal cysts leading to death in early childhood. 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 (Wanders, 2004a; Steinberg et al., 2006).

Interestingly, peroxisomes markedly enlarged in size and reduced in number have been observed in patient fibroblasts as morphological hallmarks of single enzyme deficiencies such as acyl-CoA oxidase I (AOXI) deficiency and especially of D-bifunctional protein (DBP) deficiency (Chang et al., 1999; Ebberink et al., 2010). AOXI and DBP are both important enzymes of the peroxisomal fatty acid ß-oxidation pathway, which is impaired in these deficiencies (Wanders, 2004a; Poirier et al., 2006). A loss of the enzymatic activity of AOX, fatty acyl-CoA synthetase, and/or DBP, but not the absence of these proteins, is known to cause pronounced changes in peroxisome number and/or size in mammalian cells and yeast (Poll-The et al., 1988; Fan et

al., 1998; Qi et al., 1999; Smith et al., 2000; van Roermund et al., 2000; Funato et al., 2006). These findings might point to a metabolic control of peroxisome abundance and size, e.g. by metabolic intermediates or products of peroxisomal fatty acid ß-oxidation or overproduction of other compensatory peroxisomal ß-oxidation enzymes (Smith et al., 2000; Purdue and Lazarow, 2001; Li and Gould, 2002).

Similar enlarged peroxisomes and a reduction in number have recently been reported in an unusual variant of PBD caused by mutations in PEX16 (Ebberink et al., 2010). Human Pex16p is an integral peroxisomal membrane protein with two membrane-spanning domains, which is supposed to be involved in peroxisomal membrane assembly (see 1.2.) (Honsho et al., 2002, Matsuzaki and Fujiki, 2008). Loss of Pex16p in mammalian cells is accompanied by a complete lack of peroxisomes, and reintroduction of functional PEX16 has been shown to result in the *de novo* formation of peroxisomes from the ER (Kim et al., 2006). Pex16p is supposed to travel, at least partly, via the ER to peroxisomes, and Sec16B appears to play an important role in this process (Kim et al., 2006; Yonekawa et al., 2011). PEX16-defective patients have been reported to display a severe Zellweger syndrome phenotype, and peroxisomes or membrane remnants were completely absent in patient fibroblasts (Honsho et al., 1998; Shimozawa et al., 2002). Surprisingly, six patients with mutations in the C-terminus of PEX16 have been recently identified, which displayed a relatively mild clinical phenotype with abnormal peroxisomal metabolites (e.g. raised levels of very long chain fatty acids, phytanic acid) in plasma (Ebberink et al., 2010). Patient fibroblasts unexpectedly contained peroxisomes, which were import-competent for peroxisomal matrix and membrane proteins. However, peroxisomes were increased in size but reduced in number, suggesting that Pex16p is involved in the proliferation of peroxisomes in addition to its involvement in membrane assembly. These findings are important for future diagnostics of patients with a peroxisomal disorder.

### *2.3. Peroxisomes in hepatocellular carcinomas and liver cirrhosis*

Besides Peroxisome Biogenesis Disorders and Single Enzyme Deficiencies, alterations of peroxisomes are observed in other disease conditions, e.g. in Alzheimer's Disease (Cimini et al., 2009; Kou et al., 2011; Titorenko and Terlecky, 2011). Here, we selected two classical examples, hepatocellular carcinomas and liver cirrhosis, to highlight peroxisome alterations in number.

More than a century ago Blumenthal and Brahn (1910) described a significant reduction of catalase activity in liver tumors and this notion was confirmed in subsequent studies by Greenstein (1954). After the discovery of peroxisomes as the main intracellular site of catalase activity, alterations of this organelle in neoplastic transformation were investigated and, indeed, immunocytochemical studies of human liver tumors revealed significant reductions of peroxisomal catalase as well as of all three peroxisomal ß-oxidation enzymes (Litwin et al., 1999). Interestingly, the level of reduction of peroxisomal antigens correlated well with the degree of tumor dedifferentiation, as assessed by classical histopathological criteria. Thus, poorly differentiated tumors contained no peroxisomes whereas the well differentiated adenomas exhibited almost a normal distribution of the organelle. Since all four peroxisomal proteins investigated were affected similarly, the authors suggested that the neoplastic transformation altered the biogenesis of the entire organelle and not merely an individual peroxisomal protein such as catalase (Litwin et al., 1999). Similar findings have also been reported in experimental liver tumors in rodents (Mochizuki et al., 1971; Malick, 1972; Mayer et al., 1998), suggesting that an inverse relationship exists between tumor growth rate and the abundance of hepatic peroxisomes. The exact mechanisms underlying the reduction of peroxisomes in hepatocellular carcinomas are still unknown. However, in the rat and human catalase gene several "silencer elements" have been indentified which exhibit excessive protein binding in dedifferentiated hepatoma cell lines, which are absent in normal hepatocytes, and thus have been implicated in down regulation of the catalase gene in liver tumors (Sato et al., 1992; Takeuchi et al., 2000).

In human colon carcinomas also a significant reduction of peroxisomes and their proteins catalase and acyl-CoA oxidase has been described with little alterations of their corresponding mRNAs (Lauer et al., 1999). Moreover, PEX5, a receptor protein involved in the import of most peroxisomal matrix proteins (see 1.2.) was elevated in those tumors. The authors concluded that the reduction of peroxisomes and their proteins was not due to a generalized reduction of transcription of their genes but rather due to a post-transcriptional or translational phenomenon. Alternatively, an impairment of the biogenesis of the organelle could account for the paucity of peroxisomes in colonic tumors (Lauer et al., 1999).

Another factor that could account for the down regulation of peroxisomal proteins in association with tumors and/or inflammatory processes is the cytokine tumor necrosis factor-alpha (TNF-α). Intravenous injection of recombinant TNF-α reduces significantly the levels of several peroxisomal proteins and their corresponding mRNAs (Beier et al., 1992, 1997). Since TNF-α also reduces the expression of PPAR-α, it is likely that this transcription factor may also be involved in the regulation of peroxisomal enzymes in hepatic tumors. Indeed, its role in regulation of energy combustion, hepatic steatosis, inflammation and liver cancer has been well investigated (Pyper et al., 2010).

In contrast to the reduction of peroxisomes in hepatic tumors a significant proliferation of this organelle was observed in liver cirrhosis (Litwin et al., 1999). Although earlier electron microscopic studies had

indicated a numerical increase of hepatic peroxisomes in both alcoholic hepatitis and cirrhosis (De Craemer et al., 1993), the severity of proliferation was first revealed by immunohistochemical investigation of paraffin embedded sections (Litwin et al., 1999). Although the role of the peroxidatic activity of catalase in degradation of ethanol has long been known (Thurman and Handler, 1989), more recent studies implicate the oxidative stress in activation of hepatic stellate cells and their increased collagen synthesis in the pathogenesis of liver cirrhosis (Comporti et al., 2009). The central role of peroxisomes in oxidative stress (Schrader and Fahimi, 2006a; Bonekamp et al., 2009) and the participation of the transcription factor PPAR-α in the development of hepatic cirrhosis and the associated steatosis and steatohepatitis has been well documented (Yu et al., 2003).

### *2.4. Peroxisomes and viruses*

#### 2.4.1. Peroxisomes as antiviral signalling platforms

The cellular antiviral defence in mammalian cells is normally triggered upon recognition of a viral component by a cellular element. Viral RNA (and in some cases DNA) can be detected by soluble RNA helicases that belong to the family of the RIG-I-like helicases (RLH) (Yoneyama et al., 2004). The most studied members of this family, the retinoic acid inducible gene-I (RIG-I) and the melanoma differentiation-associated gene-5 (MDA-5), interact with viral nucleic acids strictly in the cytosol, in both immune and non-immune cells, and trigger a signalling pathway that will culminate in antiviral defence (Yoneyama et al., 2004; Castanier et al., 2010; Rehwinkel et al., 2010). These two proteins recognize different characteristics within the nucleic acids, hence different viruses (Kato et al., 2008). Upon viral stimulation, these proteins undergo a conformational change, leading to their dimerization and interaction with the mitochondrial antiviral signalling adaptor (MAVS, also known as Cardif, IPS-1 and VISA), through the CARD domains of both proteins (reviewed by Moore and Ting, 2008). This leads to a signalling cascade that culminates with the induction of type-1 interferons including IFN-ß, IFN-α and IFN-stimulated genes (ISGs) that may function as direct antiviral effectors, preventing important steps in viral propagation. Activation of this pathway was shown to promote mitochondria elongation which, in turn, modulates signalling downstream from MAVS (Castanier et al., 2010). MAVS has always been assumed to localize exclusively to mitochondria (with cytosolic or ER localization completely abolishing RLH signalling). However, Dixit et al. (2010) have recently demonstrated that MAVS also localizes to peroxisomes and that viral stimulation causes alterations to peroxisome morphology, inducing elongation/tubule formation. These studies have also shown that peroxisomal and mitochondrial MAVS perform different but complementing functions within the antiviral response: while the peroxisomal MAVS induces the rapid interferon-independent expression of defence factors providing short-term protection, the mitochondria MAVS activates an interferon-dependent signalling pathway with delayed kinetics that amplifies and stabilizes the antiviral response (Dixit et al., 2010). The differential role of both organelles has very recently been questioned (Horner et al., 2011). Nevertheless, these exciting findings have revealed a new biological function for peroxisomes in antiviral innate immunity and antiviral signalling.

#### 2.4.2. Peroxisomes as replication loci for plant viruses

Some members of the plant *Tombusvirus* family make use of peroxisomal membranes to assemble their replication loci, largely altering the organelles' morphology. The Tomato bushy stunt virus (TBSV) replicates at the cytosolic domain of the peroxisomal membrane, causing extensive inwards vesiculations and forming multivesicular bodies (pMVBs). Further invaginations of the pMVBs culminate with the formation of up to several hundred spherical and ovoid smaller vesicles that are thought to be the assembly area for the viral replication machinery (Jonczyk et al., 2007; Laliberté and Sanfaçon, 2010). Pex19p, the cytosolic chaperone and import receptor for peroxisomal membrane proteins (see 1.2.), is the cellular transporter of the viral replication protein p33 to the peroxisomal membrane (Pathak et al., 2008). In the absence of peroxisomes (in cells lacking PEX19 or PEX3), the replication complex can be assembled at ER membranes (Jonczyk et al., 2007). Electron microscopy analysis revealed the sporadic proximity of these vesicles to ER-like tubular membrane structures, as well as the presence of channels connecting these vesicles to the cytosol. During pMVB formation, p33 (together with resident peroxisomal membrane proteins) relocalizes from the peroxisomal membranes to a peroxisomal endoplasmic reticulum (pER) subdomain, revealing a previously unknown peroxisome-to-pER sorting pathway in plant cells (McCartney et al., 2005; Mullen and Trelease, 2006).

#### 2.4.3. Peroxisomes are essential for viral infectivity

Although only plant viruses have been shown to assemble their replication loci at the peroxisomal membranes, the importance of peroxisomes for the life-cycle of human viruses has also been demonstrated. The human immunodeficiency virus (HIV) sorts its Nef protein to peroxisomes, a phenomenon that has been shown to be essential for the development of AIDS. Although lacking a peroxisomal targeting signal (PTS), Nef forms a complex with a PTS1-containing peroxisomal matrix protein, thioesterase II, being imported into the organelle by a piggy-back mechanism

(Cohen et al., 2000). Another medically-relevant virus, the rotavirus (RV), expresses a virion surface protein (VP4) that is targeted to the peroxisomes by its own PTS1-signal (Mohan et al., 2002). It has been suggested that the importance of peroxisomes for the infectivity of HIV and RV lays in essential lipid modifications occurring in peroxisomes (Cohen et al., 2000; Mohan et al., 2002; Titorenko and Rachubinski, 2004).

## 3. Conclusions

The key molecular machinery controlling peroxisomal morphology, dynamics and number is becoming more and more established and defined. It is likely that in the coming years many more proteins involved in peroxisome dynamics and morphology will be identified contributing to a better understanding of these complex mechanisms. There is emerging evidence that these processes are crucial for the physiology of the cell, as well as for the pathology of the organism and for host defence. Dysfunctions of peroxisome-shaping proteins result in characteristic peroxisome morphologies which can serve as valuable diagnostic indicators. While several key proteins involved in peroxisome dynamics and proliferation have been identified, their coordinated interplay and the regulation of these processes are not well understood. There is growing evidence for the role of post-translational modifications in the regulation of peroxisome morphology proteins (e.g. Pex11p, DLP1); however, the cellular signals and the precise modulation of signal transduction pathways by physiological stimuli leading to peroxisome proliferation are largely unknown. The scenario is further complicated by the fact that the key peroxisome division proteins are shared with mitochondria, pointing to organelle cross-talk, cooperative metabolism and coordinated biogenesis of both organelles (Schrader and Yoon, 2007; Camões 2009). In a very recent study, peroxisome proliferation and subsequent alterations in ROS levels have been demonstrated to set melanocortin tone and feeding in diet-induced obesity (Diano et al., 2011). These findings further highlight the importance of peroxisome proliferation and regulation of peroxisome number in disease conditions. A better molecular understanding of the peroxisome morphology machinery and the regulation of peroxisome proliferation will contribute to therapeutical approaches for the treatment of patients with defects in peroxisome biogenesis and other disorders.

**Note added in proof:** Very recently, Itoyama et al. (J. Cell Sci. 125, 589-603, 2012) reported that docosahexaenoic acid, a product of peroxisomal fatty acid beta-oxidation and a component of phospholipids, promotes peroxisome elongation and proliferation in AOX or DBP-defective fibroblasts and restores the normal peroxisome morphology.

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