Histochem Cell Biol (2004) 122:383–393
DOI 10.1007/s00418-004-0673-1

REVIEW

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Mammalian peroxisomes and reactive oxygen species

Accepted: 7 June 2004 / Published online: 8 July 2004

Abstract The central role of peroxisomes in the generation and scavenging of hydrogen peroxide has been well known ever since their discovery almost four decades ago. Recent studies have revealed their involvement in metabolism of oxygen free radicals and nitric oxide that have important functions in intra- and intercellular signaling. The analysis of the role of mammalian peroxisomes in a variety of physiological and pathological processes involving reactive oxygen species (ROS) is the subject of this review. The general characteristics of peroxisomes and their enzymes involved in the metabolism of ROS are briefly reviewed. An expansion of the peroxisomal compartment with proliferation of tubular peroxisomes is observed in cells exposed to UV irradiation and various oxidants and is apparently accompanied by upregulation of PEX genes. Significant reduction of peroxisomes and their enzymes is observed in inflammatory processes including infections, ischemia-reperfusion injury, and allograft rejection and seems to be related to the suppressive effect of tumor necrosis factor-α on peroxisome function and peroxisome proliferator activated receptor-α. Xenobiotic-induced proliferation of peroxisomes in rodents is accompanied by the formation of hepatic tumors, and evidently the imbalance in generation and decomposition of ROS plays an important role in this process. In PEX5$^{-/-}$ knockout mice lacking functional peroxisomes severe alterations of mitochondria in various organs are observed which seem to be due to a generalized increase in oxidative stress confirming the important role of peroxisomes in homeostasis of ROS and the implications of its disturbances for cell pathology.

Keywords Peroxisomes · ROS · Peroxisome proliferation · Aging · PEX5$^{-/-}$ mice

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Introduction on peroxisomes

The peroxisome is a ubiquitous organelle which is present in nearly all eukaryotic cells, including unicellular eukaryotes. Peroxisomes are morphologically characterized by a single limiting membrane and a finely granular matrix with a range in size from 0.1 to 1 μm in diameter (Fig. 1). De Duve and Baudhuin (1966) first isolated the peroxisomes and discovered that they contained H_2O_2-producing oxidases as well as catalase, an H_2O_2-degrading enzyme, and therefore coined the functional term peroxisome, which gradually replaced the former morphological designation, microbody, introduced by Rhodin (1954). In the meantime more than 50 enzymes have been discovered in mammalian peroxisomes that participate in various metabolic pathways including β-oxidation of very long ($\geq C_{24}$; VLCFA) and long (C_{14-22}) chain fatty acids, prostaglandins, and leukotrienes (Ferdinandusse et al. 2002), which are poor substrates for mitochondrial β-oxidation; biosynthesis of cholesterol, bile acids (in the liver), dolichol, and ether lipids (plasmalogens) (van den Bosch et al. 1992); oxidation of D-amino acids, polyamines, and uric acid (in non-primates) (Subramani et al. 2000; Purdue and Lazarow 2001); and detoxification of xenobiotics, glyoxylate, and last but not least reactive oxygen species (ROS). The major metabolic functions of mammalian peroxisomes are summarized in Table 1.

Most of the peroxisomal membrane and matrix proteins are synthesized on free polyribosomes in the cytoplasm and are then transported post-translationally to the organelle (Lazarow and Fujiki 1985). Sorting of peroxisomal matrix proteins is achieved by cytosolic receptors (Pex5p for PTS1 and Pex7p for PTS2) that recognize two well-characterized classes of peroxisomal targeting sig-

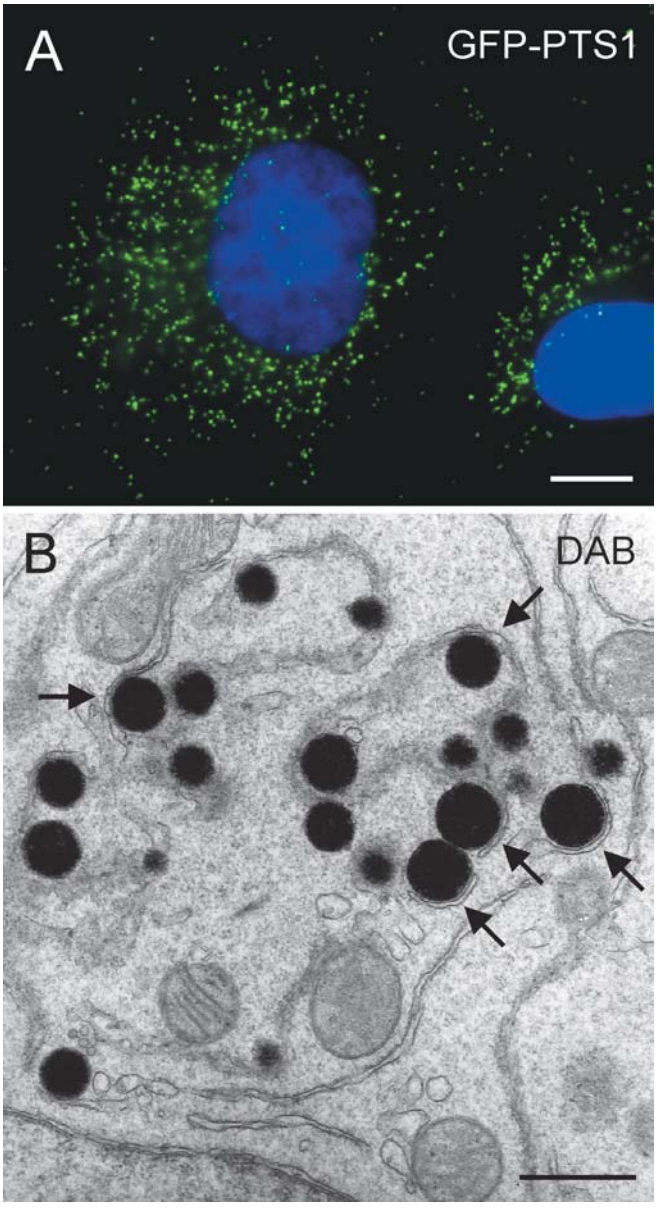


Fig. 1A, B Presentation of mammalian peroxisomes by light (**A**) and electron microscopy (**B**). **A** Fluorescence microscopy of peroxisomes in COS-7 cells. Cells were transfected with a GFP construct (*GFP-PTS1*) bearing the peroxisomal targeting signal 1 (PTS1). After 24 h cells were fixed with 4% paraformaldehyde and mounted for fluorescence microscopy. Nuclei (*blue*) were stained with Hoechst 33258. Note the uniform intracellular distribution of peroxisomes (*green*) which is maintained by microtubules in mammalian cells (Schrader et al. 2003). **B** Ultrastructure of peroxisomes in rat hepatoma cells. For cytochemical localization of catalase (Angermuller and Fahimi 1981), the cells were incubated in alkaline DAB medium followed by postfixation in 1% aqueous osmium tetroxide and 2% uranyl acetate. Notice the close association of peroxisomes (*black*) with the smooth endoplasmic reticulum (*arrows*). *Bars* 10 μm in **A**; 500 nm in **B**

nals (PTS1 and PTS2). The PTS1 comprises a C-terminal tripeptide (SKL), whereas the PTS2 is located near the N-terminus. Most of the identified *peroxins*, the proteins involved in peroxisome biogenesis (Pexp), are involved in

Table 1 Metabolic functions of mammalian peroxisomes

Metabolism of peroxide (catalase and H_2O_2-generating oxidases) and other ROS
Lipid biosynthesis [ether phospholipids (plasmalogens), bile acids, cholesterol and dolichol, fatty acid elongation]
β-Oxidation of fatty acids (very long chain fatty acids, dicarboxylic acids, branched chain fatty acids, unsaturated fatty acids, ara chidonic acid metabolism, and xenobiotic compounds)
α-Oxidation of fatty acids (phytanic acid, xenobiotic compounds)
Catabolism of amino acids
Catabolism of polyamines
Catabolism of purines
Metabolism of glyoxylate
Hexose monophosphate pathway

matrix protein import and contribute to the formation of the docking and translocation machinery at the peroxisomal membrane. It is assumed that the receptors accompany their cargo inside the peroxisomes and recycle back to the cytosol. In contrast to proteins imported into mitochondria or endoplasmic reticulum (ER), peroxisomal proteins pass through the intact peroxisomal membrane in a folded or even oligomeric state. For a review on peroxisomal import see (Gould and Collins 2002; Eckert and Erdmann 2003).

The importance of peroxisomes for human health and disease has been best exemplified by the discovery of about 20 peroxisomal disorders in man. Peroxisome biogenesis disorders, a genetically heterogeneous group of autosomal recessive disorders, are characterized by deficiency of multiple peroxisomal functions (mostly by an inability to import proteins into peroxisomes). The best-studied example is the cerebro-hepato-renal syndrome of Zellweger which is associated with extreme hypotonia, severe mental retardation, and early death (Goldfischer et al. 1973). A second group of disorders (single enzyme disorders) is characterized by deficiency of a single peroxisome function, for example in X-linked adrenoleukodystrophy (X-ALD) which is caused by a defect in the ALD protein, an ABC transporter for VLCFA (Mosser et al. 1993). Several of these inherited disorders result in death at an early age and are characterized by an accumulation of VLCFAs and a reduction of ether lipids. For a review on peroxisomal disorders see (Wanders 2000; Weller et al. 2003).

Peroxisomes are highly dynamic organelles with marked plasticity that have been shown to move in a motor protein-dependent manner along microtubules (in mammals; Schrader et al. 2003) or actin filaments (in plants and fungi; Mathur et al. 2002) throughout the cell. Peroxisome shape, size, number, and metabolic function show considerable variation among different organisms and cell types. An interesting feature of peroxisomes is their ability to proliferate and multiply, or be degraded in response to nutritional and environmental stimuli (Beier and Fahimi 1991; Reddy and Mannaerts 1994). For example, in rodents, peroxisome number and size as well as the expression of peroxisomal β-oxidation enzymes is highly increased when activators of the peroxisome proliferator activated receptors (PPARs), such as fibrates

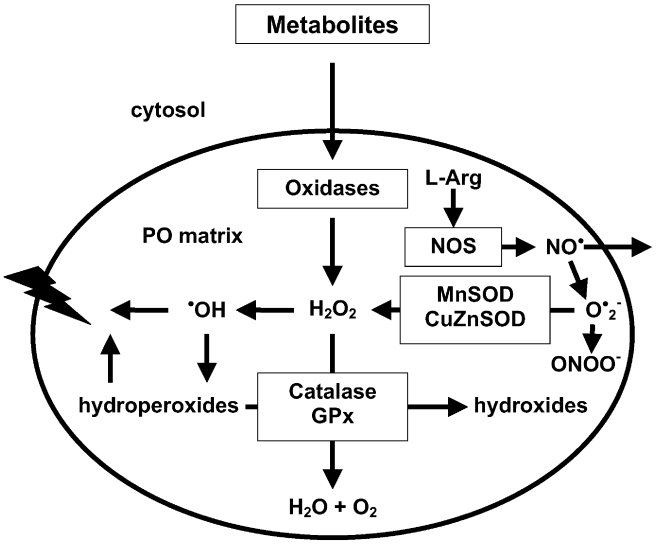


Fig. 2 Schematic overview of peroxisomal enzymes which produce or decompose reactive oxygen species (ROS). Hydrogen peroxide is produced by a number of peroxisomal oxidases (for example by acyl-CoA oxidase which is involved in the β-oxidation of fatty acids). Hydrogen peroxide is decomposed by catalase and glutathione-peroxidase (*GPx*) or converted to hydroxyl radicals. Hydroxyl radicals can damage the peroxisomal membrane by lipid peroxidation of unsaturated fatty acids. Hydroperoxides formed in this process can be decomposed by catalase and glutathione-peroxidase. Superoxide anions ($O_2^{\cdot-}$) generated by peroxisomal oxidases are scavenged by manganese superoxide dismutase (*MnSOD*) and by copper-zinc superoxide dismutase (*CuZnSOD*). Nitric oxide synthase (*NOS*) catalyses the oxidation of L-arginine (*L-Arg*) to nitric oxide (*NO*$^{\cdot}$). NO$^{\cdot}$ can react with $O_2^{\cdot-}$ radicals to form peroxynitrite (*ONOO*$^-$), a powerful oxidant

or free fatty acids, are applied (Fahimi et al. 1982; Lindauer et al. 1994).

The prevailing model of peroxisome biogenesis, proposed by Lazarow and Fujiki (1985), predicts that peroxisomes grow by uptake of newly synthesized proteins from the cytosol and multiply by division. Members of the Pex11p family of peroxisomal membrane proteins and of the dynamin family of large GTPases have recently been demonstrated to be involved in growth and division of the peroxisomal compartment (Koch et al. 2003; Li and Gould 2003; Rottensteiner et al. 2003; Tam et al. 2003). A key question in the field is, currently, whether this is the predominant mechanism of peroxisome formation, or if there are alternative modes of peroxisome formation which may involve the ER or other kinds of endomembranes (South and Gould 1999; Titorenko and Rachubinski 2001; Faber et al. 2002; Geuze et al. 2003).

There is increasing experimental evidence suggesting the involvement of peroxisomes in the metabolism of ROS including radicals and non-radical derivatives of O_2. In this review we will focus on mammalian peroxisomes, although some of the peroxisomal functions in ROS metabolism were first discovered in plant cells (del Rio et al. 2003). This review is concerned with the production and decomposition of ROS in peroxisomes (Fig. 2), the reactions of peroxisomes to oxidative stress, peroxisome proliferation, and the induction of ROS, as well as the effects of ROS in Pex5p knockout mice.

Enzymes in peroxisomes that produce ROS

The colocalization of catalase with H_2O_2-producing oxidases in peroxisomes attracted the attention of early investigators to the important role of this organelle in the metabolism of ROS (De Duve and Baudhuin 1966; Oshino et al. 1973; Sies 1974). The enzymes in mammalian peroxisomes producing H_2O_2 and $O_2^{\cdot-}$, as well as the recently discovered inducible NO synthase generating nitric oxide are compiled in Table 2. The main source of production of H_2O_2 in peroxisomes are the oxidases that transfer hydrogen from their respective substrates to molecular oxygen. Indeed, it has been estimated that about 35% of all H_2O_2 produced in rat liver is generated by peroxisomal oxidases (Boveris et al. 1972) accounting for 20% of total oxygen consumption (Reddy and Mannaerts 1994).

Acyl-CoA oxidases

The most important metabolic process associated with the generation of H_2O_2 in peroxisomes is the β-oxidation of fatty acids. The peroxisomal lipid substrates serve important physiological functions but as a source of metabolic fuel are less relevant. The various lipids are degraded by separate enzymes with distinct substrate speci-

Table 2 Enzymes in mammalian peroxisomes that generate ROS

Enzyme	Substrate	ROS
1. Acyl-CoA oxidases		
a) Palmitoyl-CoA oxidase	Long chain fatty acids	H_2O_2
b) Pristanoyl-CoA oxidase	2-Methyl branched-chain fatty acids	H_2O_2
c) Trihydroxycoprostanoyl-CoA oxidase	Bile acid intermediates	H_2O_2
2. D-Amino acid oxidase	D-Proline	H_2O_2
3. D-Aspartate oxidase	D-Aspartate, *N*-methyl-D-aspartate	H_2O_2
4. α-Hydroxyacid oxidase	Glycolate, lactate	H_2O_2
5. Pipecolic acid oxidase	L-Pipecolic acid	H_2O_2
6. Polyamine oxidase	*N*-Acetyl spermine/spermidine	H_2O_2
7. Urate oxidase	Uric acid	H_2O_2
8. Xanthine oxidase	Xanthine	$O_2^{\cdot}$-
9. NO synthase	L-Arginine	NO$^{\cdot}$

ficities (Van Veldhoven and Mannaerts 1999). Thus in rat liver there are three specific oxidases for the degradation of: (a) long and very long straight-chain fatty acids and prostaglandins; (b) 2-methyl branched-chain fatty acids; and (c) bile acid intermediates. The unique feature of this metabolic system is its inducibility via the nuclear transcription factor PPAR-α (for further details see below under peroxisome proliferation and ROS).

D-Amino acid oxidase

This flavoprotein is present in liver, kidney, and brain (Gaunt and de Duve 1976; Angermuller and Fahimi 1988). By enzyme cytochemistry marked heterogeneity in the content of D-amino acid oxidase is noted in peroxisomes of kidney and liver. The enzyme in kidney peroxisomes is confined to the central part of the matrix forming a distinct subcompartment (Yokota et al. 1987; Angermuller 1989).

D-Aspartate oxidase

This flavoenzyme oxidizes specifically the D-amino acids with two carboxylic groups such as D-aspartate, D-glutamate, and *N*-methyl-D-aspartate that have important neuroregulatory functions in the central nervous system. The enzyme has been detected in peroxisomes of brain as well as in liver and kidney (Zaar et al. 1989, 2002).

L-α-Hydroxyacid oxidase

This enzyme has two isoforms: the A isoform occurring in liver that oxidizes preferentially glycolate, and the B isoform in the kidney oxidizing best DL-α-hydroxybutyrate (Angermuller et al. 1986a, b). In kidney the enzyme is localized in the peripheral part of the peroxisome matrix, forming in some instances crystalline plate-like structures, called 'marginal plates' (Zaar et al. 1991).

Pipecolic acid oxidase

This is another flavin oxidase that catalyzes the conversion of L-pipecolic acid to piperidine-6-carboxylic acid while generating H_2O_2 (Zaar et al. 1986). The enzyme is present in human and primate peroxisomes but is mitochondrial in rabbit liver. The oxidation of L-pipecolic acid is impaired in peroxisomal deficiency disorders, although the elevation of the pipecolic acid levels is not considered as a reliable diagnostic feature (Wanders et al. 1996).

Polyamine oxidase

This enzyme oxidizes spermine and spermidine as substrates exerting an influence on the metabolism of polyamines and indirectly on the growth processes and malignant transformation (Beard et al. 1985).

Urate oxidase

This cuproprotein catalyzes the oxidation of urate, in the presence of oxygen, yielding H_2O_2, CO_2, and allantoin. Enzyme cytochemistry and immunoelectron microscopy have convincingly assigned it to the crystalline cores of rat liver peroxisomes (Angermuller and Fahimi 1986; Volkl et al. 1988). Multiple mutations of the urate oxidase gene have resulted in loss of that enzyme in humans and primates (Yeldandi et al. 1991). In view of the antioxidant actions of uric acid (Ames et al. 1981) it has been postulated that the loss of enzyme in humans may protect against diseases associated with ROS, such as aging and cancer.

Xanthine oxidase

This molybdenum-containing dimeric enzyme of the purine metabolism exists in two functionally distinct forms: type D or the dehydrogenase form, and type O or the oxidase form. Under certain conditions, such as ischemia, the NAD^+-dependent D form is converted to the O form which can cause severe tissue injury due to the generation of $O_2^{\cdot -}$ superoxide radicals and H_2O_2 (Engerson et al. 1987). The enzyme has been localized to the crystalline cores of rat liver peroxisomes (Angermuller et al. 1987).

Nitric oxide synthase

Nitric oxide is a free radical generated by NO synthase (NOS). Whereas under physiological conditions this enzyme is involved in vasodilation or signaling processes in the brain, an inducible form of it (iNOS) is expressed during inflammatory conditions and can cause severe tissue injury. In rat hepatocytes exposed to tumor necrosis factor-α, iNOS is induced and becomes detectable in peroxisomes (Stolz et al. 2002). Interestingly, the induction of iNOS is associated with marked reduction of catalase protein with the possibility of the formation of the severely toxic peroxynitrite radical.

Enzymes in peroxisomes that decompose ROS

Enzymes in mammalian peroxisomes that decompose ROS are summarized in Table 3.

Catalase

This enzyme exhibits both a catalatic and a peroxidatic activity depending upon the conditions and can metabolize, in addition to H_2O_2, a variety of substrates such as

Table 3 Enzymes in mammalian peroxisomes that degrade ROS

Enzyme	Substrate	Enzyme is also present in
1. Catalase	H_2O_2	Cytoplasm and nucleus (some cells)
2. Glutathione peroxidase	H_2O_2	All cell compartments
3. Mn SOD	$O_2^{\cdot}$-	Mitochondria
4. Cu, Zn SOD	$O_2^{\cdot}$-	Cytoplasm
5. Epoxide hydrolase	Epoxides	Cytoplasm and ER
6. Peroxiredoxin (I)	H_2O_2	Cytoplasm and nucleus

ethanol and methanol, phenols, and nitrites (Oshino et al. 1973). The inhibition of catalase can have detrimental effects upon cell survival after oxidative stress. The catalase levels are significantly reduced in tumors of the liver (Litwin et al. 1999) and other organs (Lauer et al. 1999) as well as in a variety of pathological conditions, such as liver allograft rejection (Steinmetz et al. 1998) and after ischemia-reperfusion injury (Singh 1996).

Glutathione peroxidase

In addition to its cytoplasmic presence, this enzyme has been localized to peroxisomes in rat liver (Singh et al. 1994). It can catalyze the oxidation of peroxide substrates such as cumene hydroperoxide.

Manganese superoxide dismutase (Mn SOD)

This enzyme is primarily localized in mitochondria but additionally its presence was reported in the peroxisomal membrane (Singh 1996).

Copper-zinc superoxide dismutase (Cu, Zn SOD)

The localization of this enzyme in peroxisomes of fibroblasts was reported first by Keller et al. (1991) and was subsequently confirmed for liver peroxisomes (Dhaunsi et al. 1992).

Peroxiredoxin I

This antioxidant protein has thioredoxin-dependent peroxidase activity with high binding affinity for the prooxidant heme (Iwahara et al. 1995). It belongs to the family of peroxiredoxins that have important functions in cellular protection against oxidative stress and cell signaling (Hofmann et al. 2002). In a recent immunocytochemical study the presence of peroxiredoxin I in the matrix of rat liver peroxisomes, in addition to cytoplasm and nucleus, was reported (Immenschuh et al. 2003).

Epoxide hydrolase

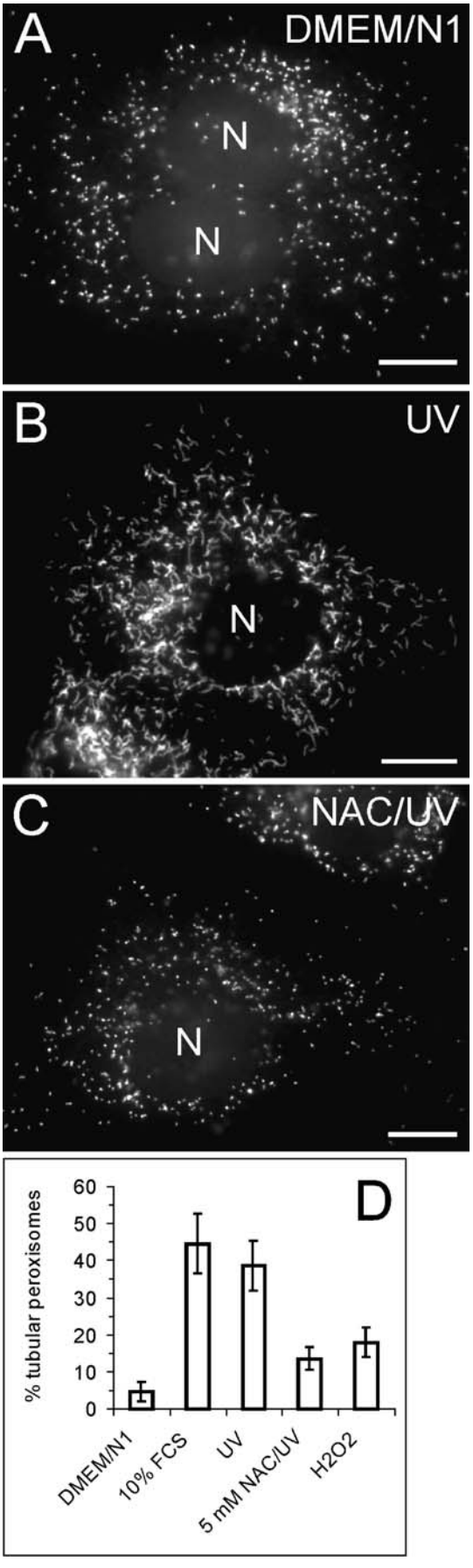
This enzyme has been localized to peroxisomes, in addition to cytoplasm and ER (Waechter et al. 1983; Pahan et al. 1996). It enables peroxisomes to convert arene or alkene oxides to dihydrodioles thus catabolizing fatty acids with an oxirane ring which are formed in sterol metabolism.

Reactions of peroxisomes to oxidative stress

After the presentation of the enzymes involved in the generation and detoxification of ROS in peroxisomes of mammalian liver, we will now deal with the reactions of peroxisomes to oxidative stress. Oxidative stress as induced by UV irradiation, direct exposure to H_2O_2, or hypoxia-reoxygenation has been shown to perturb the peroxisomal compartment with evidence of peroxisome proliferation and induction of peroxisomal genes, supporting their involvement in cellular rescue from ROS.

Cell culture studies

Microscopic observations revealed most mammalian peroxisomes in cultured cells to be spherical in shape. However, the peroxisomal compartment displays a remarkable plasticity and complexity. Several morphologically distinct types of peroxisomes, including elongated, tubular, or reticular organelles, have been described in various mammalian tissues and cell lines, both by electron microscopic studies (Hicks and Fahimi 1977; Gorgas 1984; Yamamoto and Fahimi 1987; Roels et al. 1991), as well as by light microscopy (Schrader et al. 1994, 1995; Litwin and Bilinska 1995). In live cells, the reticular peroxisomal networks appear to be extremely dynamic, with constant formation of tubular extensions interconnecting or detaching (Schrader et al. 2000). A heterogeneous and more complex peroxisomal compartment has been observed under conditions of rapid cellular growth, for example, after partial hepatectomy (Yamamoto and Fahimi 1987) or stimulation of cultured cells with defined growth factors or polyunsaturated fatty acids, particularly arachidonic acid, suggesting the involvement of intracellular signaling in peroxisome elongation (Schrader et al. 1998a). Interestingly, a pronounced formation of tubular peroxisomes was also induced in serum-free cultures of HepG2 cells by UV irradiation (Schrader et al. 1999; Fig. 3). Pretreatment with the antioxidant *N*-acetylcysteine had an inhibitory effect on UV-induced formation of tubular peroxisomes, thus supporting the role of ROS in this process. Furthermore, tubular peroxisomes could also be induced by direct administration of H_2O_2



(Fig. 3) or even during live cell imaging of GFP-PTS1-labeled peroxisomes by regular fluorescent light (unpublished observation). Although the precise mechanisms for the generation of a signal from the extracellular incidence of UV and the gene response leading to peroxisome elongation remain largely unknown, an involvement of tyrosine kinase has been suggested (Schrader et al. 1998, 1999). At present, little information is available on the exact function of complex peroxisomal structures. However, it has become evident in recent years that tubulation and fission of elongated peroxisomes contributes to peroxisome proliferation and represents processes of peroxisomal growth and division. Furthermore, tubule formation of peroxisomes seems to be an important aspect of peroxisome division (Schrader et al. 1996, 1998b). The involvement of the peroxisomal membrane protein Pex11p and the dynamin-like protein DLP1 in these processes has recently been demonstrated (Koch et al. 2003; Li and Gould 2003). Since the peroxisomes contain a variety of enzymes involved in the degradation of ROS (see Table 3; Fig. 2), the induction of tubular peroxisomes, as an indication of the growth of the peroxisomal compartment, appears to be a logical response to oxidative stress. It is likely (but has not been examined yet) that peroxisome tubulation induced by ROS is accompanied by an elevation of the antioxidative peroxisomal enzymes. Along the same line, direct exposure of plant and mammalian cells to H_2O_2 resulted in peroxisome proliferation via the upregulation of PEX genes required for peroxisome biogenesis and import of proteins (Lopez-Huertas et al. 2000). Furthermore, an elevation of peroxisomal SOD and glutathione peroxidase has been demonstrated after induction of oxidative stress by endotoxin treatment of rat liver (Dhaunsi et al. 1993). In addition, an induction of plasmalogen synthesis might be achieved by peroxisomal growth. Plasmalogens are supposed to protect against oxidative stress and to have an important anti-oxidative function in biomembranes (Nagan and Zoeller 2001). In support of this notion, a central role of peroxisomes in defense against ROS is clearly demonstrated by the high sensitivity to UV irradiation of

Fig. 3A–D UV irradiation induces tubular peroxisomes via ROS. **A–C** Immunofluorescence of HepG2 cells stained with an antibody to peroxisomal acyl-CoA oxidase. Cells were cultured in serum-free DMEM/N1 medium and treated as indicated. **A** In serum-free DMEM/N1 medium, the peroxisomes in the majority of the cells have a spherical morphology. **B** UV irradiation (*UV*) stimulates the formation of highly elongated, tubular peroxisomes. **C** Pretreatment with the antioxidant *N*-acetyl cysteine (*NAC*) prevents the induction of tubular peroxisomes by UV irradiation. *N* Nucleus. **D** Quantitative analysis of the effects on peroxisome morphology. The percentage of cells with tubular peroxisomes was determined. Whereas in serum-free DMEM/N1 medium only about 5% of the cells contain tubular peroxisomes, more than 45% contain such structures in the presence of fetal calf serum (*FCS*). Exposure to UV in the absence of FCS induces the formation of tubular peroxisomes in almost 40% of the cells (*UV*). The stimulating effect of UV irradiation on tubule formation is inhibited by pretreatment with NAC (*NAC/UV*). Hydrogen peroxide (H_2O_2) also induced, to a lesser extent, the formation of tubular peroxisomes. *Bars* 10 μm

mutant CHO cells defective in plasmalogen synthesis (Zoeller et al. 1988), and cells from patients with peroxisomal biogenesis disorders with low levels of plasmalogens (Hoefler et al. 1991; Kremser et al. 1995; Spisni et al. 1998).

Peroxisomes in inflammation, ischemia-reperfusion injury, and allograft rejection

Inflammation induces severe alterations of peroxisomal function with significant reduction of catalase activity (Canonico et al. 1975). Indeed, inflammatory cytokines such as tumor necrosis factor-α suppress the hepatic peroxisomal catalase and β-oxidation enzymes at both protein and mRNA levels (Beier et al. 1992, 1997).

Ischemia-reperfusion injury is a pathological process which is believed to be due to oxidative stress (Reilly et al. 1991). Peroxisomes from rat kidneys exposed to ischemia and reperfusion exhibited significant loss of activities of catalase and lipid β-oxidation enzymes (Gulati et al. 1992). The inhibition of peroxisomal function could lead to the accumulation of harmful metabolites of arachidonic acid (for example, eicosanoids and leukotrienes) which could further intensify the cell injury and induce inflammation (Singh 1996). Significant reductions of catalase and fatty acyl-CoA oxidase activities with structural alterations of peroxisomes were also reported in rat allogeneic liver transplantation (Steinmetz et al. 1998). The volume density and size of peroxisomes were reduced in acute rejection of liver allografts. Moreover, the suppression of catalase activity can lead to the accumulation of H_2O_2 and further inhibit the β-oxidation in peroxisomes. Indeed, experimental inhibition of catalase activity by aminotriazole has been shown to inactivate the peroxisomal thiolase and consequently suppress the entire peroxisomal lipid β-oxidation activity (Hashimoto and Hayashi 1990). Because of the central role of peroxisomes in the catabolism of inflammatory lipid mediators like leukotriene B4 (Jedlitschky et al. 1991), the impairment of peroxisomal function contributes significantly to the process of liver allograft rejection (Steinmetz et al. 1998).

Peroxisomes and aging

Oxidative stress plays an important role and contributes significantly to the process of aging by inducing the alteration and destruction of cellular macromolecular components including DNA (Ames 1989; Fraga et al. 1990; Sohal and Allen 1990). The age-dependent alterations of liver peroxisomes were investigated by Beier et al. (1993) who found significant reductions of catalase and acyl-CoA oxidase proteins with simultaneous elevations of thiolase, peroxisomal multifunctional protein, and urate oxidase. Moreover, morphometric analysis revealed a significant elevation of peroxisomal volume density in pericentral over periportal hepatocytes, in contrast to the uniform distribution of peroxisomes in the liver lobule of young male rats. By quantitative immunogold labeling it was revealed that the catalase was mainly reduced in peroxisomes of pericentral hepatocytes of aged rats that simultaneously contained increased levels of thiolase and multifunctional protein. Taken together, those alterations of peroxisomal enzymes could partly account not only for the increased production of ROS but also for the perturbations of the lipid metabolism observed in aged animals (Hegner 1980; Schnitzky 1987).

By immunoblotting, an elevation of urate oxidase protein was noted in aged rats, corresponding to an increase in core-containing peroxisomes (Beier et al. 1993). The augmentation of urate oxidase could lead to a reduction of uric acid, which is a well-known antioxidant (Ames et al. 1981), and thus contribute to the oxidative stress in aging animals. Interestingly, the peroxisomes of senescent rats respond to treatment with peroxisome proliferators by increased production of H_2O_2 but with reduced ability to decompose it because of decreased catalase activity (Badr and Birnbaum 2004). This could explain the enhanced susceptibility of senescent liver to the hepatocarcinogenic effect of peroxisome proliferating compounds.

Peroxisome proliferation and induction of ROS

One of the unique features of peroxisomes is their inducibility by xenobiotics to undergo massive proliferation usually in conjunction with increased synthesis of some of their enzymes, particularly the lipid β-oxidation enzymes (Fahimi et al. 1982; Reddy and Lalwani 1983). Since long-term treatment of rodents with most peroxisome proliferators is associated with the development of hepatic tumors, the significance of peroxisomal alterations in the pathogenesis of liver tumors and in human toxicology has attracted intensive research and debate (Moody et al. 1991). The peroxisome proliferators include structurally unrelated compounds including hypolipidemic drugs, plasticizers, lubricants, herbicides, and many toxic environmental pollutants (Beier and Fahimi 1991). The peroxisome proliferator activated receptor-α (PPAR-α), which belongs to the family of nuclear transcription factors, mediates the selective transcription of peroxisomal genes after activation by specific ligands. Whereas the expression of the genes of the lipid β-oxidation, particularly the fatty acyl-CoA oxidase is induced very strongly (10- to 30-fold depending on compound and dosage), the maximal induction of catalase does not exceed 1- to 2-fold (Fahimi et al. 1982; Rao and Reddy 1987). Since peroxisome proliferators are non-genotoxic, it has been postulated that the development of liver tumors in rodents is due to prolonged oxidative stress imposed by the disproportionate increases in H_2O_2-generating peroxisomal oxidases, particularly of the acyl-CoA oxidase and of H_2O_2-degrading catalase (Reddy and Lalwani 1983). This notion was strongly supported by in vitro studies using cultured cells transfected with cDNAs either for acyl-CoA oxidase or urate oxidase. In such transfected cells H_2O_2 production increased significantly after exposure to cor-

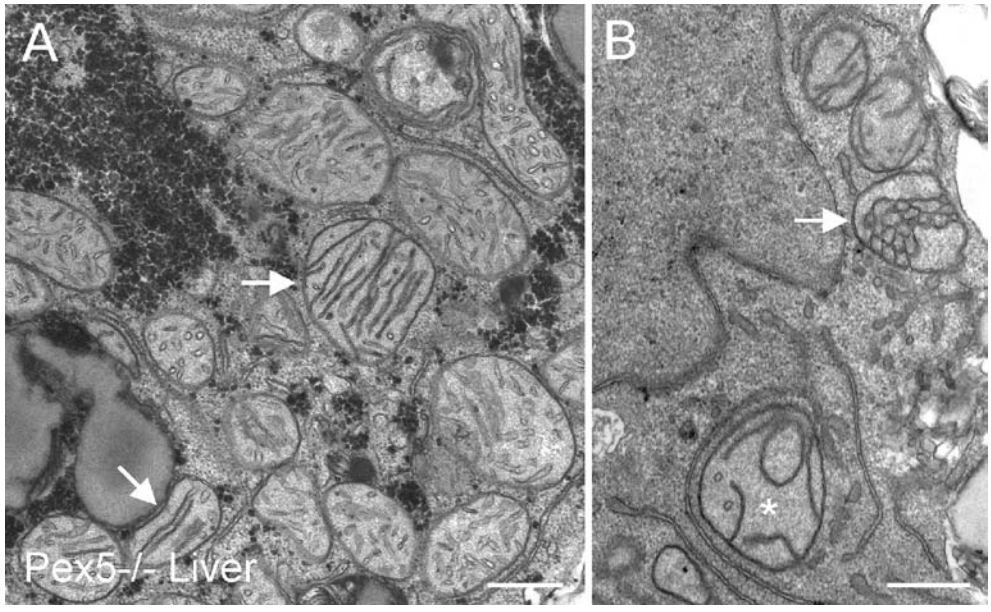


Fig. 4A, B Altered mitochondria in hepatocytes of PEX5$^{-/-}$ mice. Electron micrographs of liver sections from PEX5$^{-/-}$ mice. Note the appearance of pleomorphic mitochondria with altered cristae in **A**. Some of the round, large mitochondria exhibit stacks of parallel cristae (*arrows*). **B** Mitochondria with circular cristae (*asterisk*) or mitochondrial ghosts (*arrow*). *Bars* 500 nm

responding substrates when compared to corresponding untransfected controls. After 60 days of exposure to uric acid or the fatty acid substrate, the cells developed features of malignant transformation and formed tumors after injection to nude mice (Chu et al. 1995, 1996; Yeldandi et al. 2000). Thus, it seems that indeed peroxisomal oxidases when overexpressed in cells can lead to neoplastic transformation. The oxidative stress, however, does not seem to be the sole factor responsible for the tumor development in rodents exposed to peroxisome proliferators as additional parameters, such as potency to induce cell proliferation or to promote initiated cells, are also extremely important (Lake 1993). Moreover, additional factors, such as suppression of apoptosis (Roberts 1996), perturbation of cell proliferation, and release of superoxide radicals from Kupffer cells (Rose et al. 2000), have been suggested to play roles in the pathogenesis of hepatic tumors associated with peroxisome proliferation (Klaunig et al. 2003). The significance of Kupffer cells in this process, however, has been questioned in a very recent study (Rusyn et al. 2004).

Evidence of ROS in PEX5$^{-/-}$ mice

To study the pathogenic mechanisms causing organ dysfunctions in the cerebro-hepato-renal syndrome of Zellweger (see Introduction), a knockout mouse model was developed by disrupting the PEX5 gene, encoding the targeting receptor for most peroxisomal matrix proteins (Baes et al. 1997). PEX5$^{-/-}$ mice have a severe peroxisomal import defect, lack morphologically identifiable peroxisomes, and exhibit the typical biochemical abnormalities and pathological defects of Zellweger patients (Baes et al. 1997). Interestingly, in addition to the absence of peroxisomes, marked alterations of the mitochondria were observed in PEX5$^{-/-}$ mice (Baes et al. 1997; Baumgart et al. 2001). These alterations included the proliferation of pleomorphic mitochondria with an altered ultrastructure (Fig. 4), changes in the expression and activities of mitochondrial respiratory chain complexes, and an increase in the heterogeneity of the mitochondrial compartment in various organs and cell types (for example, liver, kidney, adrenal cortex, heart, skeletal and smooth muscle cells, and neutrophils). Furthermore, the changes of the mitochondrial respiratory chain enzymes were accompanied by a marked increase of mitochondrial Mn SOD. These findings are indicative of an increased production of ROS in altered mitochondria. Similar ultrastructural alterations have also been described in mitochondrial respiratory chain disorders (Bove 1997), and in other diseases associated with oxidative stress (Treem and Sokol 1998). It was, therefore, suggested that the defective peroxisomal antioxidant mechanisms may result in an increased oxidative stress, which in combination with the accumulation of lipid intermediates of the peroxisomal β-oxidation system, might contribute to the pathogenesis of Zellweger syndrome (Baumgart et al. 2001). Interestingly, apoptosis of neuronal cells is highly increased in the brain of the PEX5$^{-/-}$ mouse (Baes et al. 1997). It has to be elucidated whether this effect results from the accumulation of fatty acids, or is related to the defective peroxisomal antioxidant defense.

Future prospects

Because of their plasticity peroxisomes provide an ideal experimental model system for the investigation of their still numerous puzzling and enigmatic properties, including their exact role in signal transduction and disease. Moreover, with the introduction of novel techniques improved tools are now available for such future studies. Thus a new chemiluminescence method for a sensitive

and real-time determination of H_2O_2 (Mueller et al. 2002) makes possible the monitoring of the generation and degradation of H_2O_2 in suspensions of intact peroxisomes. The visualization of peroxisomes in living cells has been achieved by targeting fluorophores conjugated to peptides carrying the peroxisomal targeting signal 1 (PTS1) such as AKL (Pap et al. 2001). By using a pH-sensitive fluorescent dye linked to a PTS1 peptide, the pH in the matrix of peroxisomes was found to be in the alkaline range about 8.2 (Dansen et al. 2000). Similar studies to assess the hydroxyl-radical formation in peroxisomes have been reported (Pap et al. 1999). It would be highly interesting to use additional novel ROS-sensitive fluorophores targeted to peroxisomes and to analyze the entire spectrum of ROS and the factors influencing their formation and degradation in peroxisomes of living cells.

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