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Review

Role of peroxisomes in ROS/RNS-metabolism: Implications for human disease ☆

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ABSTRACT

Peroxisomes are cell organelles that play a central role in lipid metabolism. At the same time, these organelles generate reactive oxygen and nitrogen species as byproducts. Peroxisomes also possess intricate protective mechanisms to counteract oxidative stress and maintain redox balance. An imbalance between peroxisomal reactive oxygen species/reactive nitrogen species production and removal may possibly damage biomolecules, perturb cellular thiol levels, and deregulate cellular signaling pathways implicated in a variety of human diseases. Somewhat surprisingly, the potential role of peroxisomes in cellular redox metabolism has been underestimated for a long time. However, in recent years, peroxisomal reactive oxygen species/reactive nitrogen species metabolism and signaling have become the focus of a rapidly evolving and multidisciplinary research field with great prospects. This review is mainly devoted to discuss evidence supporting the notion that peroxisomal metabolism and oxidative stress are intimately interconnected and associated with age-related diseases. We focus on several key aspects of how peroxisomes contribute to cellular reactive oxygen species/reactive nitrogen species levels in mammalian cells and how these cells cope with peroxisome-derived oxidative stress. We also provide a brief overview of recent strategies that have been successfully employed to detect and modulate the peroxisomal redox status. Finally, we highlight some gaps in our knowledge and propose potential avenues for further research. This article is part of a Special Issue entitled: Metabolic Functions and Biogenesis of peroxisomes in Health and Disease.

1. Introduction

Over the past decades, free radicals and other reactive small molecules have emerged as important regulators of many physiological and pathological processes [1,2]. Currently, it is well established that – at physiological low levels – reactive oxygen species (ROS) and reactive nitrogen species (RNS) serve as signaling messengers to mediate various biological responses, including gene expression, cell proliferation, angiogenesis, innate immunity, programmed cell death, and senescence [3,4]. On the other hand, it is also known that increased levels of these short-lived reactive molecules can exert harmful effects by causing oxidative damage to biological macromolecules and disrupting the cellular reduction–oxidation (redox) balance [3,5]. A disturbance of ROS/RNS homeostasis is generally considered as a risk factor for the initiation and progression of diseases such as atherosclerosis, diabetes, neurodegeneration, and cancer [5,6]. Whether the effects of ROS/RNS are beneficial or harmful depends on the site, type, and amount of ROS/RNS production and the activity of the organism's antioxidant defense system [7].

Endogenous ROS/RNS can be generated as the primary function of an enzyme system (e.g. NADPH oxidases that are activated in response to activated receptors), as a byproduct of other biological reactions (e.g. the mitochondrial electron transport chain), or by metal-catalyzed oxidations (e.g. the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$) [8]. The primary ROS/RNS species generated in a cell are superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and nitric oxide ($NO^{\bullet}$) [2]. These molecules can readily react to form other ROS and RNS species. For example, $O_2^{\bullet -}$ can rapidly react with $NO^{\bullet}$ to form peroxynitrite ($ONOO^-$) or dismutate to form H_2O_2, and the latter compound can be decomposed through the Fenton reaction leading to the generation of hydroxyl radicals ($^{\bullet}OH$) [8].

To counteract oxidative and nitrosative stress, cells employ a large panel of enzymatic and non-enzymatic defense mechanisms [8]. Stress occurs when the net flux of ROS/RNS production exceeds the

Abbreviations: 3-AT, 3-Amino-1,2,4-triazole; CFP, cyan fluorescent protein; EC number, Enzyme Commission number; ER, endoplasmic reticulum; FAO, fatty acyl-CoA oxidase; FRET, fluorescence resonance energy transfer; GSH, reduced glutathione; GSSG, oxidized glutathione; NFKB1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; PPARα, peroxisome proliferator-activated receptor alpha; PTS, peroxisomal targeting signal; PTS1, C-terminal peroxisomal targeting signal; RNS, reactive nitrogen species; roGFP, redox-sensitive green fluorescent protein; ROS, reactive oxygen species; YFP, yellow fluorescent protein

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capacity of the cell to detoxify these potentially injurious oxidants [9]. It is often assumed that mitochondria are the primary source of oxidative stress in mammalian cells [10]. However, there is currently no convincing experimental evidence to support this postulate [11]. In addition, the only currently available study comparing the relative ROS production by different cellular sources demonstrated that the endoplasmic reticulum (ER) and peroxisomes may even have a greater capacity to produce ROS than mitochondria, at least in rat liver [12]. Research efforts on ER-generated oxidative stress have revealed that the production of ROS is closely linked to protein folding [13]. Furthermore, it has been shown that chronic ER-derived oxidative stress plays a critical role in the initiation of apoptosis and the pathogenesis of multiple metabolic and neurodegenerative diseases [14]. Surprisingly, for a long time, little attention was paid to the importance of peroxisomes in cellular ROS/RNS homeostasis. However, in recent years, peroxisomal ROS/RNS metabolism and signaling has become an exciting and rapidly evolving multidisciplinary research field [15–17]. In this review, we will provide an overview of our current knowledge on how peroxisomes contribute to the maintenance of cellular ROS/RNS levels and how cells cope with peroxisome-derived oxidative stress. In addition, we will highlight the present gaps in our knowledge and outline a set of strategies that can drive this research field forward. Note that we will mainly focus on mammalian peroxisomes, although it should be emphasized that a significant portion of the pioneering work in this area was carried out in other organisms such as yeasts [18], nematodes [19], and plants [16].

2. Peroxisomal ROS/RNS metabolism

The term 'peroxisome' was introduced by Nobel Laureate Christian de Duve in 1965 to define a cell organelle which contains at least one H_2O_2-producing oxidase and catalase, a H_2O_2-degrading enzyme [20]. This implies that peroxisomes generate ROS as an integral feature of their normal metabolism. This is further exemplified by the fact that peroxisomes in rat liver may be responsible for as much as 20% of the oxygen consumption and 35% of the H_2O_2 production [12,21]. Another indication that peroxisomes may act as endogenous stress generators comes from the finding that a long-term administration of peroxisome proliferators to rodents induces oxidative stress in liver cells [22]. This is most likely due to the fact that these compounds, which activate the nuclear receptor PPARα (peroxisome proliferator-activated receptor alpha), lead to a many-fold induction of H_2O_2-producing enzymes without a concomitant increase in catalase activity [23]. Numerous observations indicate that peroxisomes can also protect cells from oxidative stress. For example, the absence of functional peroxisomes causes increased apoptosis in the developing mouse cerebellum [24]; human patients suffering from an inherited deficiency of catalase, the most abundant peroxisomal antioxidant enzyme, face an increased risk of developing age-related diseases including diabetes, atherosclerosis, and cancer [25]; and mammalian cells defective in the biosynthesis of plasmalogens, a lipid species with antioxidant activity of which the two initial steps of biosynthesis are exclusively catalyzed by peroxisomal enzymes [26], are much more sensitive to ROS generated by UV-irradiation than control cells [27,28]. These observations, among others [15,29], support the idea that peroxisomal metabolism and cellular oxidative stress are closely intertwined. In the following two sections, we will focus on the major peroxisomal sources of ROS/RNS (see Section 2.1.) as well as the organelle's antioxidant defense systems (see Section 2.2.).

2.1. Peroxisomes as a cellular source of ROS/RNS species

Mammalian peroxisomes play a key role in various metabolic pathways, including fatty acid α- and β-oxidation, ether-phospholipid biosynthesis, glyoxylate metabolism, amino acid catabolism, polyamine oxidation, and the oxidative part of the pentose phosphate pathway [30]. Interestingly, many of the enzymes participating in these pathways generate specific ROS or RNS as byproducts of their normal catalytic function (Table 1) [31]. In this section, we will focus on the different types of ROS/RNS that can be generated inside peroxisomes.

2.1.1. Hydrogen peroxide

Peroxisomes contain various enzymes that produce H_2O_2 as part of their normal catalytic cycle. These enzymes, which are mainly flavoproteins, include acyl-CoA oxidases, urate oxidase, D-amino acid oxidase, D-aspartate oxidase, L-pipecolic acid oxidase, L-α-hydroxyacid oxidase, polyamine oxidase, and xanthine oxidase. For a detailed description of these enzymes, we refer to an excellent recent review covering this topic [31].

2.1.2. Superoxide- and nitric oxide radicals

Peroxisomes contain two potential enzymatic sources of $O_2^{\bullet -}$ and NO•, namely xanthine oxidase and the inducible form of nitric oxide synthase. Xanthine oxidase (XDH) is an enzyme that produces H_2O_2 (see Section 2.1.1.) and $O_2^{\bullet -}$ as byproducts of its catalytic cycle [32]. In addition, this enzyme can also reduce nitrates and nitrites to NO• [33]. The inducible form of nitric oxide synthase (NOS2) is a homodimeric enzyme that catalyzes the oxidation of L-arginine to NO• and citrulline in a complex reaction requiring O_2, NADPH, tetrahydrobiopterin (BH4), FMN, and FAD [16]. Interestingly, in the absence of adequate substrate or when in its monomeric form, the enzyme can also produce significant amounts of $O_2^{\bullet -}$ [34]. The precise subcellular localization of NOS2 remains enigmatic and can vary depending on the cell type and the environment of the cell [35]. Localization studies of NOS2 have shown that this protein displays a dual cytosolic-peroxisomal localization in hepatocytes [35,36]. Interestingly, although the molecular mechanisms underlying NOS2 targeting to peroxisomes remain to be determined, it has been shown that the peroxisomal pool of NOS2 mainly consists of 'inactive' monomers while the cytosolic pool is composed of both monomers and 'active' homodimers [36]. These findings, in combination with the observation that monomeric NOS2 can generate $O_2^{\bullet -}$, led to the hypothesis that NOS2 might be sequestered by peroxisomes to protect the larger cellular environment from monomeric NOS2-generated superoxide [36]. Nevertheless, at the moment, it cannot be rigorously excluded that – under certain circumstances – peroxisomal NOS2 may also actively produce NO• and function as a source of RNS signaling molecules.

2.1.3. Hydroxyl radical and peroxynitrite

Currently, there is no evidence that mammalian peroxisomes contain enzymes that produce •OH or $ONOO^-$. However, as already mentioned in the Introduction, H_2O_2 inside peroxisomes may give rise to •OH through the Fenton reaction. In addition, as (i) these organelles contain enzymatic sources of $O_2^{\bullet -}$ and NO• (see Section 2.2.2.), and (ii) the reaction of NO• with $O_2^{\bullet -}$ to form $ONOO^-$ is kinetically and thermodynamically favored [37], it is very likely that peroxisomes also generate $ONOO^-$.

2.2. The peroxisomal antioxidant defense system

Mammalian peroxisomes contain various ROS metabolizing enzymes, including catalase, superoxide dismutase 1, peroxiredoxin 5, glutathione S-transferase kappa, 'microsomal' glutathione S-transferase, and epoxide hydrolase 2 (Table 2). In addition, there is some evidence that these organelles also employ non-enzymatic low molecular weight antioxidant compounds. Each of these antioxidant defense systems will be discussed in more detail below.

2.2.1. Catalase

Catalase (CAT) is a homotetrameric heme-containing enzyme that can remove H_2O_2 in a catalatic ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$) or peroxidatic

Table 1
Human peroxisomal enzymes that produce ROS/RNS as byproducts of their normal catalytic activity.

Name	Protein symbol	EC number	ROS/RNS	PTS	Localization
Acyl-CoA oxidase 1	ACOX1	1.3.3.6	H_2O_2	PTS1	PO
Acyl-CoA oxidase 2	ACOX2	1.3.3.6	H_2O_2	PTS1	PO
Acyl-CoA oxidase 3	ACOX3	1.3.3.6	H_2O_2	PTS1	PO
D-amino acid oxidase	DAO	1.4.3.3	H_2O_2	PTS1	PO
D-aspartate oxidase	DDO	1.4.3.1	H_2O_2	PTS1	PO
L-pipecolic acid oxidase	PIPOX	1.5.3.1	H_2O_2	PTS1	PO
L-α-hydroxyacid oxidase 1	HAO1	1.1.3.15	H_2O_2	PTS1	PO
L-α-hydroxyacid oxidase 2	HAO2	1.1.3.15	H_2O_2	PTS1	PO
Polyamine oxidase	PAOX	1.5.3.13	H_2O_2	PTS1	PO
Xanthine oxidase	XDH	1.17.1.4	H_2O_2, NO•, $O_2•^-$	U	PO/C/MT
Inducible nitric oxide synthase	NOS2	1.14.13.39	NO•, $O_2•^-$	U	C/PO

C, cytosol; MT, mitochondria; PO, peroxisomes; U, unknown.

($H_2O_2 + AH_2 \rightarrow A + 2\ H_2O$) manner. Potential hydrogen donors (AH_2) include – among others – alcohols, formate, and nitrite [38]. Mammalian catalase can also act as an oxidase, using O_2 when H_2O_2 is absent [39]. Among the substrates identified for this oxidase action are indole, β-phenylethylamine, and various carcinogens and anticarcinogens [38]. Catalase is one of the most abundant peroxisomal proteins within mammalian cells. It contains a non-canonical C-terminal peroxisomal targeting signal (PTS1) and is targeted to the organelle by PEX5, the PTS1-import receptor [40]. However, in certain cell types or under certain conditions, catalase may also be (partially) localized to the cytosol and the nucleus [41,42]. The exact physiological function of catalase is not yet completely understood. Its predominant role is most likely to prevent the accumulation of toxic levels of H_2O_2. This may in turn prevent the formation of hydroxyl radicals by the Fenton reaction. The oxidatic and peroxidatic activities of catalase may serve to metabolize and/or detoxify small molecular weight electron donors. For example, it is well-established that in brain, the catalase-mediated oxidation of ethanol is an important source of acetaldehyde, a compound involved in the behavioral and neurotoxic effects of ethanol in humans [43,44]. Intriguingly, despite these apparently important functions, catalase activity is not essential for life. Indeed, catalase null mice develop normally and do not display any gross physical or behavioral abnormalities [45]. Nevertheless, tissues from these mice show a differential sensitivity to oxidant injury and exhibit a retarded rate in consuming extracellular H_2O_2 [45].

2.2.2. Superoxide dismutase 1

Superoxide dismutase 1 (SOD1) is a homodimeric enzyme that can convert $O_2•^-$ to O_2 and H_2O_2 ($2\ O_2•^- + 2\ H^+ \rightarrow O_2 + H_2O_2$). The protein, which can also be found in the cytosol, the nucleus, and mitochondria, is imported into peroxisomes via a piggyback mechanism in complex with 'copper chaperone for SOD1', a PTS1-containing physiological interaction partner [46]. Currently, it is postulated that SOD1 and catalase comprise a short metabolic route protecting cells from damage by ROS [31]. In addition, as $O_2•^-$ can rapidly react with NO• to form $ONOO^-$, SOD1 has been suggested to play an important role in the reduction of nitrosative stress [47]. Interestingly, mutations in the SOD1 gene account for approximately 20–25% of the patients with familial amyotrophic lateral sclerosis [48]. It has also been shown that transgenic mice containing an extra copy of the human SOD1 gene display a similar phenotype to Down syndrome, including neurological defects and premature aging [49].

2.2.3. Peroxiredoxin 5

Peroxiredoxin 5 (PRDX5) is a thiol-dependent monomeric peroxidase that can reduce H_2O_2 to H_2O, alkyl hydroperoxides (ROOH) to their respective alcohols (ROH), and $ONOO^-$ to nitrite (ONO^-) [50]. The reducing equivalents needed for these reactions are thought to be provided by thioredoxins (TRXs), a group of small, multifunctional proteins that act as antioxidants by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange [51]. Interestingly, human PRDX5 has been shown to react much faster with $ONOO^-$ ($\sim 10^7\ M^{-1}\ s^{-1}$) and ROOH ($\sim 10^6\ M^{-1}\ s^{-1}$) than with H_2O_2 ($\sim 10^5\ M^{-1}\ s^{-1}$) [52]. In addition, this enzyme has been found in different subcellular compartments including the cytosol, the nucleus, mitochondria and peroxisomes [53]. Currently, it is well-established that mammalian PRDX5 is targeted to peroxisomes by virtue of a PTS1 targeting signal [54]. The exact function of this protein inside peroxisomes is not yet known. On one hand, it is thought that PRDX5 may assist catalase in the removal of H_2O_2. However, based on the measured rate constants, it is more likely that the enzyme functions in the detoxification process of peroxynitrite and lipid peroxides [50]. Finally, it should be noted that the physiological electron donor for PRDX5 in the peroxisomal matrix remains to be identified.

2.2.4. Glutathione S-transferase kappa and microsomal glutathione S-transferase 1

Glutathione S-transferases (GST) are a superfamily of enzymes that catalyze the conjugation of xenobiotics and potentially damaging oxidative metabolites with glutathione [55]. Currently, there is some evidence that both glutathione S-transferase kappa (GSTK1) and 'microsomal' glutathione S-transferase 1 (MGST1) are at least partially located in peroxisomes [56,57]. Both enzymes have also been found in mitochondria, and – as can be deduced from its name – MGST1 is also present in the ER [58,59]. The peroxisomal localization of GSTK1, which is a soluble homodimeric enzyme, depends on a PTS1 sequence [56]. How the membrane-bound homotrimeric protein MGST1 is targeted to peroxisomes is not yet known [57]. The exact functions of GSTK1 and MGST1 within peroxisomes remain to be established. However, based on their in vitro activities, it has been suggested that these proteins may play an important role in the detoxification of xenobiotic compounds and lipid peroxide products [31,57].

2.2.5. Epoxide hydrolase 2

Epoxide hydrolase 2 (EPHX2) is a homodimeric enzyme that can bind epoxides and convert them to the corresponding dihydrothiols [60]. The protein, found in both the cytosol and peroxisomes, contains a relatively weak PTS1 targeting signal [61]. Currently, it is thought that the main physiological role of EPHX2 is to detoxify fatty-acid derived epoxides [62]. Interestingly, certain polymorphisms in the human gene are associated with an increased risk of atherosclerosis and cardiovascular diseases [63].

2.2.6. Low molecular weight antioxidants

Free radicals can also be scavenged by non-enzymatic low molecular weight antioxidants. These compounds, which are either water-

Table 2
Human peroxisomal antioxidant enzymes.

Name	Protein symbol	EC number	Substrate	PTS	Localization
Catalase	CAT	1.11.1.6	H_2O_2	PTS1	PO/C?
Superoxide dismutase 1	SOD1	1.15.1.1	$O_2•^-$	PM	C/MT/PO/N
Peroxiredoxin 5	PRDX5	1.11.1.15	$ONOO^-$, ROOH, H_2O_2	PTS1	C/MT/PO/N
GST kappa 1	GSTK1	2.5.1.18	U	PTS1	PO
Microsomal GST 1	MGST1	2.5.1.18	U	U	ER/MT/PO
Epoxide hydrolase 2	EPHX2	3.3.2.10	Epoxides	PTS1	PO/C

C, cytosol; ER, endoplasmic reticulum; GST, glutathione S-transferase; MT, mitochondria; N, nucleus; PM, piggyback mechanism (for more information, see Section 2.2.2.); PO, peroxisomes; U, unknown (for more information, see Section 2.2.4.).

or lipid-soluble, are synthesized within the body or supplemented by diet [64]. The low molecular weight antioxidants that will be discussed in more detail below include glutathione, ascorbic acid, and plasmalogens.

Glutathione, a cysteinyl tripeptide, is one of the most prevalent and important redox buffers of the cell [65]. The molecule can exist in either a reduced (GSH) or oxidized (GSSG) state. GSH, the biologically active form, can participate in numerous redox reactions and is oxidized to GSSG during oxidative and nitrosative stress conditions [7]. GSSG is reduced back to GSH by an NADPH-dependent glutathione reductase (GR, EC 1.8.1.7). Currently, it is widely accepted that the GSH/GSSG ratio reflects the redox capacity of a cell [66]. Nevertheless, it is also clear that the GSH/GSSG redox couple functions in conjunction with redox proteins such as TRXs and glutaredoxins (GRXs) [5]. GSH is exclusively synthesized in the cytosol, from where it is transferred into other cellular compartments [65]. Whether or not peroxisomes contain a functional glutathione redox system is not yet clear. For example, there is some evidence that GSH may freely penetrate the peroxisomal membrane through PXMP2, a non-selective pore-forming protein with an upper molecular size limit of 300–600 Da [67]. In addition, it has been shown that roGFP2, a genetically-encoded probe that senses changes in the glutathione redox potential via GRX [68], responds quickly and reversibly to redox changes in the peroxisomal matrix (for more details, see Section 3.1.1.) [69]. However, it remains to be investigated how oxidized roGFP2 and GSSG are reduced inside the peroxisomal matrix, and – in case the organelles would completely lack glutathione reductase activity – how GSSG is exported back into the cytosol.

Ascorbic acid (vitamin C) is a water-soluble dietary supplement that can act as cofactor and antioxidant [70]. In peroxisomes, the compound has been reported to be necessary for maximal activity of phytanoyl-CoA 2-hydroxylase, a peroxisomal α-oxidation enzyme that catalyzes the 2-hydroxylation of 3-methyl-branched acyl-CoAs [71]. Whether or not ascorbic acid also has an antioxidant function inside mammalian peroxisomes is less clear. Indeed, it has recently been demonstrated that cultivating mammalian cells in the presence of ascorbic acid actually results in an increased redox state of the peroxisomal matrix [69]. This may be explained by the combined findings that (i) peroxisomes contain relatively large amounts of heme- and non-heme iron-containing enzymes [72], and (ii) ascorbic acid can reduce transition metals, and this may in turn lead to the generation of free radicals through the Fenton reaction [70].

Another interesting group of non-enzymatic antioxidants may be plasmalogens. Indeed, albeit the physiological function of this specific class of glycerolipids is still poorly understood, there is currently good evidence that these molecules – which are produced from peroxisome-derived intermediates – may act as radical scavengers [26]. For example, it has been shown that plasmalogens are able to protect unsaturated membrane lipids against oxidation by singlet oxygen without producing oxidation products that are excessively toxic [73]. In addition, cells deficient in peroxisome assembly and/or plasmalogen biosynthesis are several orders of magnitude more sensitive to UV-induced ROS production than control cells [27].

3. Detection and modulation of the peroxisomal redox state

Over the years, various experimental model systems have been used to gain a better insight into the physiological role of peroxisomal ROS/RNS metabolism and signaling. In this section, we will provide an overview of the strategies that have been successfully employed in the past. In addition, we will highlight some recent advances in the research area of oxidative stress and discuss how these developments may open new avenues in the field of peroxisome research. In this context, it is also interesting to note that, although peroxisomes are densely populated with oxidative enzymes, these organelles maintain a reduced redox environment [69,74]. Whether or not this environment is more reducing than that of the cytosol or mitochondria depends on the cell type (e.g. fibroblasts versus pancreatic β-cells) and culture conditions (e.g. low or high concentrations of ascorbic acid, serum, and/or glucose) ([69]; Lemaire and Fransen, unpublished results).

3.1. Detection of peroxisomal ROS/RNS

During the past decade, many probes have been developed to measure oxidative stress in both live and fixed cells. These probes can largely be grouped into three different classes: (i) compounds and reagents that can be used to detect irreversible oxidative modifications (e.g. protein carbonylation) [2]; (ii) chemical fluorescent and luminescent probes for the detection of reactive oxygen and nitrogen species [75]; and (iii) proteinaceous reporter molecules [76]. Here we will focus in more detail on the genetically-encoded fluorescent imaging probes which have recently gained a lot of interest for multiple reasons: (i) they offer the possibility to append organelle-specific targeting signals and to perform noninvasive compartment-specific measurements; (ii) they are reversible and can be used to quantify transient redox changes; and (iii) they are ratiometric by excitation, thus minimizing measurement errors resulting from probe concentration, probe distribution, and cell thickness [77]. These are all important requirements to detect ROS/RNS with a high degree of spatial and temporal resolution.

3.1.1. Redox-sensitive green fluorescent proteins

Redox-sensitive green fluorescent proteins (roGFPs) are currently the most commonly used probes for monitoring the organellar redox state in living cells. Members of this class of proteins contain engineered cysteine residues on adjacent surface-exposed β-strands that form a disulfide bond under oxidizing conditions [78]. As oxidation of the dithiol pair causes reciprocal changes in emission intensity when excited at ~400 and ~490 nm, the ratio of roGFP emissions (at ~510 nm) can provide a non-destructive read-out of the redox environment of the fluorophore [77,78]. RoGFPs preferentially interact with GRXs, indicating that these probes most likely equilibrate with the local glutathione redox potential [79]. As distinct subcellular compartments often have different redox environments, various roGFPs with different reduction potentials of the disulphide have been developed [68,80]. We have recently shown that roGFP2 is a suitable probe to monitor redox changes in the peroxisomal matrix in living cells [69].

3.1.2. HyPer

HyPer is a ratiometric probe specifically developed to detect H_2O_2 [81]. The protein, which consists of a circularly permutated yellow fluorescent protein inserted into the regulatory domain of a prokaryotic H_2O_2-sensing protein, contains two cysteine residues that are specifically oxidized by H_2O_2, but not by $O_2^{\bullet -}$, $ONOO^-$, and GSSG [82,83]. HyPer exhibits two excitation peaks at ~420 and ~500 nm and one emission peak at ~516 nm. Rise in H_2O_2 concentration results in a decrease in the 420 nm excitation peak and a proportional increase in the 500 nm excitation peak [81]. Interestingly, the half maximal effective concentration of HyPer for H_2O_2 is ± 8 μM, which is almost 25 times less than that of roGFP [83]. A potential disadvantage of this probe is that it is rather pH-sensitive [81]. Recently, HyPer has been used to demonstrate that H_2O_2 produced inside peroxisomes is an important mediator of lipotoxicity in insulin-producing cells [84].

3.1.3. Redoxfluor

Redoxfluor is a recently developed fluorescence resonance energy transfer (FRET)-based probe that can sense the redox potential of glutathione via its internal disulfide bonds [74]. The protein contains a tandem repeat of a partial region within the carboxy-terminal cysteine-rich domain of Yap1, a transcription factor crucial for oxidative stress response in *Saccharomyces cerevisiae*, and mediates FRET between cerulean, a variant of the cyan fluorescent protein (CFP),

and citrine, a yellow fluorescent protein (YFP) derivative [85]. Upon oxidation, CFP emission is enhanced at the expense of YFP emission, thereby decreasing the yellow-to-cyan emission ratio (527/476 nm). Recently, this probe has been successfully used to develop an efficient screening system for redox modulators that can restore the redox status in mammalian cell lines with defective peroxisome assembly without affecting the redox status of normal cells [74].

3.2. Modulation of the peroxisomal redox state

To gain a better insight into how cells respond to peroxisome-derived oxidative stress, it is essential to have access to model systems in which the peroxisomal redox status can be selectively modulated in a controllable manner. Here we will discuss some of the strategies that have already been successfully used to manipulate peroxisome-derived oxidative stress in mammalian cells. Note that, although these studies clearly indicate that peroxisomes may constitute a cellular source of ROS/RNS, none of these strategies has yet been employed to study the corresponding cellular responses at the molecular level.

3.2.1. Peroxisome proliferators and fatty acids

Already more than a decade ago, several studies reported that the peroxisomal redox balance can be disturbed by the selective activation of peroxisomal metabolism. For example, after Reddy and colleagues discovered that the administration of peroxisome proliferators to rodents can induce liver cancer [86], they also studied the effect of such a treatment on oxidative stress and found a disproportionate increase in H_2O_2-producing enzymes and catalase [22,87]. Other studies showed that (i) mammalian cells stably overexpressing peroxisomal fatty acyl-CoA oxidase (FAO) became neoplastic upon exposure to a fatty acid substrate for 2–6 weeks [88,89], (ii) a transient overexpression of peroxisomal FAO in Cos-1 cells led to nuclear NFKB1 DNA binding activity in a dose-dependent manner [90], and (iii) a chronic exposure of pancreatic β-cells to long-chain non-esterified fatty acids increased peroxisomal H_2O_2-production, ultimately leading to β-cell death [91]. In summary, these findings demonstrate that it is technically possible to induce peroxisomal oxidative stress in intact cells by stimulating the organelle's metabolism.

3.2.2. Catalase activity

As catalase is the most abundant antioxidant enzyme in mammalian peroxisomes, it represents an attractive target to modulate the organelle's antioxidative stress system in intact cells. Several completely different approaches have already been used for this purpose. Perhaps the most straightforward way is to incubate the cells with 3-amino-1,2,4-triazole (3-AT), a well-characterized irreversible inhibitor of catalase [92]. At non-cytotoxic dosages, such a treatment may decrease catalase activity by ±70% [93]. Alternatively, one may employ genetically modified cells (e.g. from transgenic or knockout mice) [45,94], vector-driven expression systems [69,95], or even cell-penetrating catalase derivatives [96,97]. In this context, it is interesting to mention that both reducing and increasing catalase activity may make cells more vulnerable to different types of oxidative stress [94,96]. These findings indicate that nonlethal concentrations of H_2O_2 may exert net beneficial effects on the cell, and that catalase overexpression may interfere with the potential signaling mechanisms of H_2O_2 [94]. The effects of altered catalase activities on human health will be discussed in Section 6.

3.2.3. Other (potential) strategies

Another strategy that has already been successfully employed to selectively produce oxidative stress inside peroxisomes is the use of KillerRed, a genetically-encoded photosensitizer which produces radicals and H_2O_2 upon green light illumination [98,99]. By using a peroxisomal variant of this protein, we recently found that excessive ROS production inside peroxisomes may disturb the mitochondrial redox balance, and this can in turn lead to mitochondrial fragmentation [69]. An alternative method that may be better suited for biochemical investigations are transgenic cells overexpressing D-amino acid oxidase, a bona fide peroxisomal matrix protein [100]. Indeed, a nuclear targeted version of this protein has already successfully been used to study the effects of nuclear localized oxidative stress [101]. In addition, it is most likely easier to control the production of H_2O_2 in a D-amino acid-regulatable system than in a peroxisome proliferator- or free fatty acid-dependent system. Finally, one may consider altering the activities of other peroxisomal antioxidant enzymes. However, as most of these enzymes are located in multiple cellular compartments (see Section 2.2.), it is virtually impossible to study the peroxisome-specific effects on the cellular phenotype by knocking down their expression (e.g. through RNA interference). Nevertheless, one may solve this problem, at least partially, by artificially targeting these enzymes to peroxisomes by the use of a strong PTS1 (see Section 4.3.2).

4. Targets of peroxisomal ROS/RNS

Peroxisomes may potentially function as an intracellular source of H_2O_2, $O_2^{\bullet -}$, •OH, NO•, and $ONOO^-$ (see Section 2.1.). These small reactive molecules have distinct biological properties resulting from their chemical reactivity, half-life, and lipid solubility (Table 3). For example, the •OH radical is highly reactive and therefore reacts very close to the site of its production. The half-life of H_2O_2 is much longer, and this molecule has preferred biological targets [102]. The range of ROS/RNS action is co-determined by their free aqueous diffusion distances [103]. This distance may vary depending on the action of ROS/RNS scavengers, their solubility in lipids, and the membrane permeability of the organelle in which these molecules are produced. In this context, it is important to note that peroxisomes are densely populated with antioxidant enzymes (see Section 2.2.), and that the peroxisomal membrane contains porin-like channels which may facilitate the transit of ROS/RNS from the peroxisomal matrix to the cytosol (and vice versa) [67].

4.1. Peroxisomal matrix proteins

Oxidative and nitrosative stress can directly lead to reversible or irreversible protein modifications. For example, H_2O_2 may oxidize cysteine sulfhydryl groups to form disulfide bridges, sulfenic acids, or sulfinic acids; NO• may react with sulfhydryls to yield S-nitrosothiols; and other redox-based protein modifications may include hydroxylations, carbonylations, nitrosylations, and the destruction of iron–sulfur clusters [2].

Previous studies have shown that chronically reducing catalase activity in mammalian cells to approximately 38% of normal, increased the levels of carbonylated proteins, and particularly in the peroxisome-enriched organelle fraction [93]. This may be a surprising observation given that H_2O_2, the substrate of catalase, is not particularly reactive toward biological molecules. However, as transition metals such as iron are abundantly present in peroxisomes, the accumulation of H_2O_2 most likely results in the generation of highly reactive hydroxyl radicals via the Fenton reaction (see Section 1.). Note that protein carbonylation is considered to be an irreversible posttranslational modification [2], and the effects of this modification on peroxisome function are not yet clear. However, in order to preserve organelle function, these oxidatively damaged proteins are most likely proteolytically removed by the peroxisomal Lon protease [104,105].

It is also thought that, within the peroxisomal matrix, NO• can combine with $O_2^{\bullet -}$ to form $ONOO^-$, a strong oxidizing and nitrating agent that may inactivate peroxisomal enzymes [16,35]. For example, NO• and $ONOO^-$ can rapidly react with the heme group of catalase and inactivate the enzyme [16]. This may in turn promote overproduction of

H_2O_2. $ONOO^-$ may also react with peroxisomal GSH to form S-nitrosoglutathione (GSNO), a long distance signal molecule. It has been hypothesized that peroxisomal GSNO may act as a powerful inducer of defense genes in plants [16]. The potential function of peroxisomal GSNO in mammalian cells, if any, is not yet clear.

4.2. The peroxisomal membrane

Currently, it is widely accepted that polyunsaturated fatty acids, which are important lipid constituents in biological membranes, are key targets for strong oxidants such as •OH and $ONOO^-$ [106]. The corresponding damage, called lipid peroxidation, may have profound effects on membrane fluidity, membrane permeability, and the activity of membrane-bound enzymes and receptors [3]. In addition, lipid peroxidation breakdown products (e.g. 4-hydroxynonenal) may act as second messengers of oxidative stress in autophagy, apoptosis, and cell proliferation signaling pathways [106]. At the moment, surprisingly little is known on the potential role of oxidative stress-induced lipid peroxidation in mammalian peroxisomes. Nevertheless, it is tempting to speculate that peroxisomes with oxidatively damaged membranes are selectively removed by autophagy. How this may occur, remains to be investigated.

4.3. The extraperoxisomal environment

Early studies with isolated rat liver peroxisomes have revealed that 20–60% of the H_2O_2 generated inside peroxisomes diffuses to the surrounding medium [12]. This finding demonstrates that H_2O_2 can rapidly cross the peroxisomal membrane, most likely through the recently identified porin-like channel [67]. In addition, a more recent study has shown that – in intact rat liver peroxisomes – H_2O_2 generated by the core-localized urate oxidase is directly released in the cytoplasm via crystalloid core tubules [107]. Together, these findings indicate that peroxisomes are not able to prevent the release of intraperoxisomal H_2O_2, despite their high content of catalase. As these organelles also contain enzymatic sources of membrane-permeant NO• (see Section 2.2.2.), it is highly likely that – at least under certain physiological or pathological conditions – peroxisomes may act as a cellular source of both H_2O_2 and NO• in living cells. Nevertheless, very little is currently known about how, and to what extent, these peroxisome-derived small reactive molecules contribute to cellular redox homeostasis and redox signaling. NO• is thought to act through its primary receptor, guanylyl cyclase, which upon activation produces cyclic GMP, another second messenger [1]. Other intracellular targets of this reactive nitrogen species may include Fe^{2+}-containing proteins and reactive protein thiols [108]. H_2O_2 is thought to signal through the chemoselective oxidation of deprotonated cysteine residues in target proteins [109]. In addition, this molecule may react with Fe^{2+}-containing cofactors found in a select set of proteins [109]. In this section, we will focus on how peroxisome-derived oxidative stress may activate various redox-sensitive signaling pathways. For a link between peroxisomal ROS/RNS production and human disease, we refer to Section 6.

Table 3
Chemical properties of ROS/RNS produced in peroxisomes.[a]

Name	Formula	Biological half-life[b]	Membrane-permeability[c]
Hydrogen peroxide	H_2O_2	~10^{-5} s	Very low
Superoxide radical	$O_2^{\bullet -}$	~10^{-6} s	Very low
Hydroxyl radical	•OH	~10^{-9} s	Very low
Nitric oxide radical	NO•	<1 s	High
Peroxynitrite	$ONOO^-$	~1 s	Very low

[a] These data were compiled from [37,83,103,158–160].
[b] These values may be strongly influenced by local antioxidant activities.
[c] These results have been obtained with artificial lipid membranes.

4.3.1. Peroxisomal ROS production and nuclear gene expression

The intracellular localization and activity of numerous signaling proteins and transcription factors are, directly or indirectly, controlled by the oxidation of thiol groups of redox-sensitive cysteine residues [9,110]. For example, it has been shown that some transcription factors (e.g. nuclear factor (erythroid-derived 2)-like 2) possess reversibly oxidizable cysteines in their active site, and that – upon exposure to ROS – their sulfhydryl groups are oxidatively modified, which may cause their nuclear translocation and lead to enhanced cytoprotective gene expression [111,112]. Currently, there is strong evidence that peroxisomal H_2O_2 may function as an important modulator of NFKB1, a pleiotropic transcription factor that is involved in many biological processes, including inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis [113]. For example, it has been reported that a transient overexpression of the H_2O_2-producing peroxisomal enzyme FAO resulted in a concentration-dependent DNA binding activity of NFKB1 DNA in Cos-1 cells, and that this phenomenon could be counteracted by overexpression of catalase [90]. In addition, it has been shown that a genetic or pharmacological inactivation of catalase in mouse neutrophils inhibited the nuclear accumulation of NFKB1 and the production of the proinflammatory cytokines TNF-α and MIP-2 [114]. Note that, to make sense of these apparently conflicting data, it is important to know that – depending on the cell type and the experimental conditions – H_2O_2 may stimulate or inhibit the NFKB1 activation pathway [115]. Despite this, these data clearly show that the production of H_2O_2 inside peroxisomes can affect nuclear gene expression. As the peroxisomal localization of NOS2 has been associated with a reduced expression level of catalase in hepatocytes [35], the same might be true for peroxisomal NO• production. However, note that – as (i) only a fraction of NOS2 is localized in peroxisomes (see Section 2.1.2.), and (ii) it is not yet clear whether or not peroxisomal NOS2 effectively produces NO• (see Section 2.2.2.) – this remains to be investigated in more detail.

4.3.2. Redox communication between peroxisomes and mitochondria

Peroxisomes and mitochondria exhibit a functional interplay that continues to emerge [116,117]. Over the past years, it has become clear that these organelles also share a redox-sensitive relationship. For example, it has been shown that catalase-SKL, a catalase derivative with enhanced peroxisome targeting, can efficiently repolarize mitochondria in late passage cells [118]. In addition, we recently found that the mitochondrial redox balance is disturbed in cells lacking catalase or functional peroxisomes, and upon the generation of excess ROS inside peroxisomes [69; Apanasets and Fransen, unpublished results]. On the other hand, peroxisomes were found to resist oxidative stress generated inside mitochondria [69]. In summary, these findings suggest that peroxisome-derived oxidative stress may trigger signaling/communication events that ultimately result in increased mitochondrial stress. The molecular mechanisms underlying this phenomenon remain unclear. A number of molecules may participate in linking these organelles. For example, peroxisomes initiate oxidative metabolism of specific fatty acids which are then trafficked to mitochondria where processing is completed [119]. The organelles also communicate via anaplerotic metabolism [17]. It can be envisioned that as peroxisomal metabolism is slowed down, critical metabolic intermediates (e.g. acetyl-CoA) are not properly produced and trafficked to mitochondria [69]. If mitochondrial metabolism is similarly disrupted, an increase in uncoupled reactions and ROS production can certainly be anticipated. There also exists the mitochondrial retrograde signaling pathway, a process involving multiple factors that sense mitochondrial dysfunction and transmit signals to the nucleus to affect gene expression [120]. The protein products of these genes promote, among other processes, peroxisomal β-oxidation and peroxisome proliferation [17]. Finally, the organelles may also interact via reactive oxygen species.

5. Peroxisomes as targets of oxidative stress

Currently, little is known about how peroxisomes cope with oxidative stress. In this section, we will discuss the pros and cons of these organelles as a sink for cellular ROS as well as how cellular ROS may affect peroxisome biology and function.

5.1. Peroxisomes as a sink for cellular ROS

The very high content of catalase inside peroxisomes led to the experimentally-based prediction that these organelles may serve as an intracellular sink for H_2O_2 [121]. This prediction is in line with the findings that hypocatalasemic fibroblasts accumulate H_2O_2 and are oxidatively damaged [122], and that overexpression of catalase in pancreatic islets of transgenic mice produced a marked protection of islet insulin secretion against H_2O_2 [123]. However, as already discussed in Section 4.3., other studies strengthen the notion that peroxisomes rather represent a potential source of oxidative stress, which may cause damage to the cell or modulate redox-sensitive pathways [107,124]. Taken together, these apparently conflicting observations illustrate that caution must be taken when studying the potential contribution of peroxisomal ROS metabolism to cellular redox homeostasis. In this context, it may also not be that difficult to envision that this contribution may display strong plasticity under different physiological and pathological conditions.

5.2. Effects of cellular ROS on peroxisome biology and function

Currently, there is substantial evidence that peroxisome number and morphology can drastically change upon exposure of cells to various conditions of oxidative stress. For example, both UV-irradiation and a pharmacological depletion of cellular GSH induce extensive peroxisome elongation in mammalian cells [15,29]. In addition, it has been reported that the number of peroxisomes drastically increases while cells age, a process associated with an increase in systemic oxidative stress [42,125]. The underlying mechanisms and the functional significance of these findings are still largely unknown. However, it has been established that the PEX5-mediated import pathway into peroxisomes is – at least partially – functionally impaired in cells experiencing oxidative stress conditions [42,126]. This manifests itself primarily in the accumulation of PEX5 at the peroxisomal membrane and a partial mislocalization of catalase to the cytosol [42]. As (i) monoubiquitination of PEX5 at a conserved cysteine residue (e.g. Cys11 in human PEX5) is a requisite for its ATP-dependent export back into the cytosol [127], and (ii) shifting the cytosolic redox balance back to a more reduced state restores PTS1 import in late passage cells (Apanasets and Fransen, unpublished results), it is tempting to speculate that the Cys11 in PEX5 may act as a functional redox switch thereby regulating the peroxisomal/cytosolic localization of peroxisomal proteins such as catalase. Such a mechanism may allow cells to rapidly respond to oxidative stress in the cytosol [128]. With respect to the relationship between peroxisome number and oxidative stress, it has been suggested that cellular aging may somehow alter the regulation of peroxisome growth and division, leading to organelle proliferation in the absence of normally required cellular cues [42]. However, as mammalian peroxisomes are mainly degraded via macroautophagy [129–131] and the rate of autophagy slows with age [132], it can currently not be ruled out that the accumulation of peroxisomes in aging cells is an indirect result of reduced autophagy.

6. Peroxisomal ROS metabolism and human disease

It is currently a common belief that alterations in the cellular redox state impose a considerable risk for the development of various diseases [6]. As (i) the intracellular redox status is inherently linked to cellular metabolism, and (ii) peroxisomes regulate major fluxes of primary and secondary metabolites, it may not be surprising to see that also these organelles are increasingly recognized as being involved in human pathologies related to oxidative stress. In this context, it is interesting to note that – for example – compromised catalase activity has already been associated with ischemia-reperfusion injury, hypertension, skin pigmentation disorders, retinal disease, degenerative joint disease, heart failure, type 2-diabetes, neurodegenerative disorders, and the initiation and progression of certain cancers ([93]; and references therein). In addition, the protein and activity levels of this enzyme have recently been shown to be downregulated in nasopharyngeal secretions of infants with naturally acquired respiratory syncytial virus infections [133]. In this section, we will highlight the potential role of peroxisomal oxidative stress in the pathogenesis of neurodegeneration, diabetes, aging, and cancer.

6.1. Neurodegeneration

In recent years, it has become increasingly clear that alterations in peroxisome function may contribute to the development of neurodegenerative diseases [134]. This is best illustrated by pathomorphological examinations of the brain of patients (and mice) in which one or more peroxisomal functions are lost [30,134–137]. The major neuropathological features of these disorders include impaired neuronal migration, axonal degeneration, and progressive subcortical demyelination [136–141]. The mechanisms underlying these phenotypes, which most likely result from a combination of facts, have not yet been elucidated [134]. One of these pathogenic factors may be oxidative stress. Indeed, it has been reported that mitochondrial SOD2, a marker for oxidative stress, is significantly upregulated in different organs of peroxisome-deficient mice [142]. In addition, it has been shown that peroxisomes provide oligodendrocytes with neuroprotective and anti-inflammatory functions [138], and that these cells protect neuronal axons from the harmful effects of H_2O_2 [143]. Finally, it has been demonstrated that a cocktail of antioxidants can halt and even reverse axonal damage in a mouse model of X-linked adrenoleukodystrophy, a demyelinating peroxisomal disorder [140].

6.2. Type 2-diabetes

For some time, it is known that elevated levels of long-chain and very-long-chain saturated non-esterified fatty acids exhibit a strong cytotoxic effect on insulin-producing β-cells [91,144]. Interestingly, these cells contain virtually no catalase [145,146], and an elegant study has recently demonstrated that the cytotoxic phenomenon – referred to as lipotoxicity – is mediated by H_2O_2 derived from peroxisomal β-oxidation [84]. Importantly, overexpression of catalase in the peroxisomes and the cytosol, but not in the mitochondria, protected the cells against palmitic acid-induced toxicity [84]. In summary, these experiments led to a completely new concept in the pathogenesis of fatty acid-induced lipotoxicity for pancreatic β-cells.

6.3. Aging

More and more evidence indicates that peroxisomes may also play a vital role in the chronic processes of cellular and organismal aging. Many studies on this topic have focused on the role of catalase. Nonetheless, the results are – albeit indicative – not always straightforward. For example, detailed epidemiological studies on patients suffering from hypocatalasemia revealed an increased frequency of age-related diseases, including – among others – type 2-diabetes, hypertension, and vitiligo [147]. Despite this, mice completely lacking catalase develop normally and are apparently healthy [45]. In addition, long-lived dwarf mice and short-lived transgenic mice exhibit increased and decreased levels of catalase, respectively [148]. However, transgenic mice overexpressing peroxisomal catalase do not show

a significant increase in lifespan [149]. Interestingly, mice overexpressing catalase targeted to the mitochondrial matrix display an increased life expectancy by ±20% [149]. Finally, inactivation of catalase activity has a negative impact on the mitochondrial redox balance [69,93], a condition commonly associated with aging [150]. However, cellular aging may also compromise peroxisomal ROS metabolism [42]. Importantly, a retroviral-mediated transduction of catalase-SKL cDNA in aging cells reduced the cellular H_2O_2 levels and restored mitochondrial membrane polarization [118]. In summary, these studies suggest that peroxisomes may play a physiological role in aging. In addition, the finding that alterations in the peroxisomal ROS metabolism can affect the mitochondrial redox balance [69,93,118], may have important implications for how we think about aging and age-related diseases. In this context, it has recently been suggested that peroxisomal ROS may not only provoke pro-aging effects, but also function as anti-aging signaling molecules, akin to mitochondrial ROS [17].

6.4. Cancer

Currently, there is growing evidence that peroxisomes may be either directly or indirectly involved in cancer development. For example, the administration of nongenotoxic peroxisome proliferators to rodents can induce liver cancer [86,151]; hypocatalasemic mice display a higher susceptibility to spontaneous and induced tumorigenesis [152,153]; and peroxisome number and catalase activity are significantly reduced in various tumors, including colon, hepatocellular and renal cell carcinoma [154–156]. The precise mechanism of how peroxisomes may contribute to the cancer process is not well understood. One may envision that peroxisomal ROS/RNS may cause permanent oxidative damage to the cell's genetic material, which in turn may lead to genomic instability and carcinogenesis. Alternatively, increased peroxisomal oxidative stress may – akin to mitochondrial ROS – lead to the aberrant induction of stress-sensitive signaling pathways that cause tumorigenesis [5].

7. Conclusions and future directions

Over the last decade, peroxisomes have emerged as potentially important players of cellular redox metabolism. Strong arguments have been presented that – depending on the physiological and pathological situation – these organelles may function as a source, a sink, or a target of small reactive molecules. In addition, compelling evidence support a direct relationship between peroxisomes and ROS/RNS signaling. For example, high levels of peroxisomal ROS may invoke profound changes in gene expression; peroxisomes may act as an upstream initiator of mitochondrial ROS signaling pathways; and alterations in peroxisomal redox metabolism have been associated with the etiology and progression of age-related diseases, perhaps best exemplified by cancer and type 2-diabetes. An intriguing question that remains to be answered is how excessive levels of peroxisomal ROS/RNS can lead to the activation of such stress-sensitive signaling pathways. The molecular details underpinning these responses are just beginning to emerge. However, in analogy to mitochondria [157], it is tempting to speculate that peroxisomal ROS/RNS may play a significant role in the maintenance and/or regulation of the cellular disulfide proteome. In this context, it is interesting to note that cultivating bovine aortic endothelial cells in the presence of 3-AT increased cellular protein disulfide content by 20% [157]. Future studies should focus on the identification of the proximal targets of peroxisomal ROS/RNS as well as on the molecular mechanisms of how cellular ROS/RNS impinge on peroxisome function. Such studies may provide a powerful route toward a more coherent understanding of the mechanisms and pathways that mediate the relationship between peroxisomes and oxidative stress. This, in turn, is crucial to gain a better insight into the physiological relevance of these organelles in cellular aging and the initiation and progression of age-related diseases.

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References

[1] D.D. Thomas, L.A. Ridnour, J.S. Isenberg, W. Flores-Santana, C.H. Switzer, S. Donzelli, P. Hussain, C. Vecoli, N. Paolocci, S. Ambs, C.A. Colton, C.C. Harris, D.D. Roberts, D.A. Wink, The chemical biology of nitric oxide: implications in cellular signaling, Free Radic. Biol. Med. 45 (2008) 18–31.
[2] C. Nathan, A. Ding, SnapShot: reactive oxygen intermediates (ROI), Cell 140 (2010) 951-951.
[3] D.K. Dowling, L.W. Simmons, Reactive oxygen species as universal constraints in life-history evolution, Proc. Biol. Sci. 276 (2009) 1737–1745.
[4] R. Scherz-Shouval, Z. Elazar, Regulation of autophagy by ROS: physiology and pathology, Trends Biochem. Sci. 36 (2011) 30–38.
[5] A. Acharya, I. Das, D. Chandhok, T. Saha, Redox regulation in cancer: a double-edged sword with therapeutic potential, Oxid. Med. Cell. Longev. 3 (2010) 23–34.
[6] A.B. Salmon, A. Richardson, V.I. Pérez, Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? Free Radic. Biol. Med. 48 (2010) 642–655.
[7] M.L. Circu, T.Y. Aw, Reactive oxygen species, cellular redox systems, and apoptosis, Free Radic. Biol. Med. 48 (2010) 749–762.
[8] S.K. Powers, M.J. Jackson, Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production, Physiol. Rev. 88 (2008) 1243–1276.
[9] K.E. Wellen, C.B. Thompson, Cellular metabolic stress: considering how cells respond to nutrient excess, Mol. Cell 40 (2010) 323–332.
[10] A.A. Starkov, The role of mitochondria in reactive oxygen species metabolism and signaling, Ann. N. Y. Acad. Sci. 1147 (2008) 37–52.
[11] G.C. Brown, V. Borutaite, There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells, Mitochondrion. 12 (2012) 1–4.
[12] A. Boveris, N. Oshino, B. Chance, The cellular production of hydrogen peroxide, Biochem. J. 128 (1972) 617–630.
[13] C.X. Santos, L.Y. Tanaka, J. Wosniak, F.R. Laurindo, Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase, Antioxid. Redox Signal. 11 (2009) 2409–2427.
[14] J.D. Malhotra, R.J. Kaufman, Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid. Redox Signal. 9 (2007) 2277–2293.
[15] N.A. Bonekamp, A. Völkl, H.D. Fahimi, M. Schrader, Reactive oxygen species and peroxisomes: struggling for balance, Biofactors 35 (2009) 346–355.
[16] L.A. Del Río, Peroxisomes as a cellular source of reactive nitrogen species signal molecules, Arch. Biochem. Biophys. 506 (2011) 1–11.
[17] V.I. Titorenko, S.R. Terlecky, Peroxisome metabolism and cellular aging, Traffic 12 (2011) 252–259.
[18] A. Mesquita, M. Weinberger, A. Silva, B. Sampaio-Marques, B. Almeida, C. Leão, V. Costa, F. Rodrigues, W.C. Burhans, P. Ludovico, Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing hydrogen peroxide and superoxide dismutase activity, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 15123–15128.
[19] O.I. Petriv, R.A. Rachubinski, Lack of peroxisomal catalase causes a progeric phenotype in *Caenorhabditis elegans*, Biol. Chem. 279 (2004) 19996–20001.
[20] C. de Duve, Functions of microbodies (peroxisomes), J. Cell Biol. 27 (1965) 25A.
[21] C. de Duve, P. Baudhuin, Peroxisomes (microbodies and related particles), Physiol. Rev. 46 (1966) 323–357.
[22] H. Kasai, Y. Okada, S. Nishimura, M.S. Rao, J.K. Reddy, Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term exposure to a peroxisome proliferator, Cancer Res. 49 (1989) 2603–2605.
[23] J.K. Reddy, S.K. Goel, M.R. Nemali, J.J. Carrino, T.G. Laffler, M.K. Reddy, S.J. Sperbeck, T. Osumi, T. Hashimoto, N.D. Lalwani, M.S. Rao, Transcription regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 1747–1751.
[24] O. Krysko, L. Hulshagen, A. Janssen, G. Schütz, R. Klein, M. De Bruycker, M. Espeel, P. Gressens, M. Baes, Neocortical and cerebellar developmental abnormalities in conditions of selective elimination of peroxisomes from brain or from liver, J. Neurosci. Res. 85 (2007) 58–72.
[25] L. Góth, J.W. Eaton, Hereditary catalase deficiencies and increased risk of diabetes, Lancet 356 (2000) 1820–1821.
[26] S. Wallner, G. Schmitz, Plasmalogens the neglected regulatory and scavenging lipid species, Chem. Phys. Lipids 164 (2011) 573–589.
[27] R.A. Zoeller, O.H. Morand, C.R. Raetz, A possible role for plasmalogens in protecting animal cells against photosensitized killing, J. Biol. Chem. 263 (1988) 11590–11596.

[28] G. Hoefler, E. Paschke, S. Hoefler, A.B. Moser, H.W. Moser, Photosensitized killing of cultured fibroblasts from patients with peroxisomal disorders due to pyrene fatty acid-mediated ultraviolet damage, J. Clin. Invest. 88 (1991) 1873–1879.
[29] M. Schrader, H.D. Fahimi, Peroxisomes and oxidative stress, Biochim. Biophys. Acta 1763 (2006) 1755–1766.
[30] R.J. Wanders, H.R. Waterham, Peroxisomal disorders: the single peroxisomal enzyme deficiencies, Biochim. Biophys. Acta 1763 (2006) 1707–1720.
[31] V.D. Antonenkov, S. Grunau, S. Ohlmeier, J.K. Hiltunen, Peroxisomes are oxidative organelles, Antioxid. Redox Signal. 13 (2010) 525–537.
[32] S. Angermüller, G. Bruder, A. Völkl, H. Wesch, H.D. Fahimi, Localization of xanthine oxidase in crystalline cores of peroxisomes. A cytochemical and biochemical study, Eur. J. Cell Biol. 45 (1987) 137–144.
[33] R. Harrison, Structure and function of xanthine oxidoreductase: where are we now? Free Radic. Biol. Med. 33 (2002) 774–797.
[34] D. Stuehr, S. Pou, G.M. Rosen, Oxygen reduction by nitric-oxide synthases, J. Biol. Chem. 276 (2001) 14533–14536.
[35] D.B. Stolz, R. Zamora, Y. Vodovotz, P.A. Loughran, T.R. Billiar, Y.M. Kim, R.L. Simmons, S.C. Watkins, Peroxisomal localization of inducible nitric oxide synthase in hepatocytes, Hepatology 36 (2002) 81–93.
[36] P.A. Loughran, D.B. Stolz, Y. Vodovotz, S.C. Watkins, R.L. Simmons, T.R. Billiar, Monomeric inducible nitric oxide synthase localizes to peroxisomes in hepatocytes, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13837–13842.
[37] P. Pacher, J.S. Beckman, L. Liaudet, Nitric oxide and peroxynitrite in health and disease, Physiol. Rev. 87 (2007) 315–424.
[38] H.N. Kirkman, G.F. Gaetani, Mammalian catalase: a venerable enzyme with new mysteries, Trends Biochem. Sci. 32 (2007) 44–50.
[39] A.M. Vetrano, D.E. Heck, T.M. Mariano, V. Mishin, D.L. Laskin, J.D. Laskin, Characterization of the oxidase activity in mammalian catalase, J. Biol. Chem. 280 (2005) 35372–35381.
[40] P.E. Purdue, P.B. Lazarow, Targeting of human catalase to peroxisomes is dependent upon a novel COOH-terminal peroxisomal targeting sequence, J. Cell Biol. 134 (1996) 849–862.
[41] K. Yamamoto, A. Völkl, H.D. Fahimi, Investigation of peroxisomal lipid beta-oxidation enzymes in guinea pig liver peroxisomes by immunoblotting and immunocytochemistry, J. Histochem. Cytochem. 40 (1992) 1909–1918.
[42] J.E. Legakis, J.I. Koepke, C. Jedeszko, F. Barlaskar, L.J. Terlecky, H.J. Edwards, P.A. Walton, S.R. Terlecky, Peroxisome senescence in human fibroblasts, Mol. Biol. Cell 13 (2002) 4243–4255.
[43] N. Oshino, R. Oshino, B. Chance, The characteristics of the "peroxidatic" reaction of catalase in ethanol oxidation, Biochem. J. 131 (1973) 555–563.
[44] M.M. Goyal, A. Basak, Human catalase: looking for complete identity, Protein Cell 1 (2010) 888–897.
[45] Y.S. Ho, Y. Xiong, W. Ma, A. Spector, D.S. Ho, Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury, J. Biol. Chem. 279 (2004) 32804–32812.
[46] M. Islinger, K.W. Li, J. Seitz, A. Völkl, G.H. Lüers, Hitchhiking of Cu/Zn superoxide dismutase to peroxisomes — evidence for a natural piggyback import mechanism in mammals, Traffic 10 (2009) 1711–1721.
[47] T. Fukai, M. Ushio-Fukai, Superoxide dismutases: role in redox signaling, vascular function, and diseases, Antioxid. Redox Signal. 15 (2011) 1583–1606.
[48] J.S. Valentine, P.A. Doucette, S. Zittin Potter, Copper–zinc superoxide dismutase and amyotrophic lateral sclerosis, Annu. Rev. Biochem. 74 (2005) 563–593.
[49] C.J. Epstein, K.B. Avraham, M. Lovett, S. Smith, O. Elroy-Stein, G. Rotman, C. Bry, Y. Groner, Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in Down syndrome, Proc. Natl. Acad. Sci. U. S. A. 84 (1984) 8044–8048.
[50] B. Knoops, J. Goemaere, V. Van der Eecken, J.P. Declercq, Peroxiredoxin 5: structure, mechanism, and function of the mammalian atypical 2-cys peroxiredoxin, Antioxid. Redox Signal. 15 (2011) 817–829.
[51] Y. Meyer, B.B. Buchanan, F. Vignols, J.P. Reichheld, Thioredoxins and glutaredoxins: unifying elements in redox biology, Annu. Rev. Genet. 43 (2009) 335–367.
[52] M. Trujillo, A. Clippe, B. Manta, G. Ferrer-Sueta, A. Smeets, J.P. Declercq, B. Knoops, R. Radi, Pre-steady state kinetic characterization of human peroxiredoxin 5: taking advantage of Trp84 fluorescence increase upon oxidation, Arch. Biochem. Biophys. 467 (2007) 95–106.
[53] V. Van der Eecken, A. Clippe, P.P. Van Veldhoven, B. Knoops, Mitochondrial targeting of peroxiredoxin 5 is preserved from annelids to mammals but is absent in pig Sus scrofa domesticus, Mitochondrion 11 (2011) 973–981.
[54] H. Yamashita, S. Avraham, S. Jiang, R. London, P.P. Van Veldhoven, S. Subramani, R.A. Rogers, H. Avraham, Characterization of human and murine PMP20 peroxisomal proteins that exhibit antioxidant activity in vitro, J. Biol. Chem. 274 (1999) 29897–29904.
[55] A. Sala, M. Lanciotti, M.G. Valsecchi, P. di Michele, C. Dufour, R. Haupt, G. Basso, C. Rizzari, A. Biondi, Genotypes of the glutathione S-transferase superfamily do not correlate with outcome of childhood acute lymphoblastic leukemia, Leukemia 17 (2003) 981–983.
[56] F. Morel, C. Rauch, E. Petit, A. Piton, N. Theret, B. Coles, A. Guillouzo, Gene and protein characterization of the human glutathione S-transferase kappa and evidence for a peroxisomal localization, J. Biol. Chem. 279 (2004) 16246–16253.
[57] M. Islinger, G.H. Lüers, H. Zischka, M. Ueffing, A. Völkl, Insights into the membrane proteome of rat liver peroxisomes: microsomal glutathione-S-transferase is shared by both subcellular compartments, Proteomics 6 (2006) 804–816.
[58] I.R. Jowsey, R.E. Thomson, T.C. Orton, C.R. Elcombe, J.D. Hayes, Biochemical and genetic characterization of a murine class Kappa glutathione S-transferase, Biochem. J. 373 (2003) 559–569.
[59] K. Johansson, J. Järvliden, V. Gogvadze, R. Morgenstern, Multiple roles of microsomal glutathione transferase 1 in cellular protection: a mechanistic study, Free Radic. Biol. Med. 49 (2010) 1638–1645.
[60] M. Decker, M. Arand, A. Cronin, Mammalian epoxide hydrolases in xenobiotic metabolism and signaling, Arch. Toxicol. 83 (2009) 297–318.
[61] R.T. Mullen, R.N. Trelease, H. Duerk, M. Arand, B.D. Hammock, F. Oesch, D.F. Grant, Differential subcellular localization of endogenous and transfected soluble epoxide hydrolase in mammalian cells: evidence for isozyme variants, FEBS Lett. 445 (1999) 301–305.
[62] S. Summerer, A. Hanano, S. Utsumi, M. Arand, F. Schuber, E. Blée, Stereochemical features of the hydrolysis of 9,10-epoxystearic acid catalysed by plant and mammalian epoxide hydrolases, Biochem. J. 366 (2002) 471–480.
[63] Y.X. Wang, A. Ulu, L.N. Zhang, B. Hammock, Soluble epoxide hydrolase in atherosclerosis, Curr. Atheroscler. Rep. 12 (2010) 174–183.
[64] T. Grune, P. Schröder, H.K. Biesalski, Low molecular weight antioxidants, Handb. Environ. Chem. 2 (2005) 77–90.
[65] M. Kemp, Y.M. Go, D.P. Jones, Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology, Nat. Rev. Mol. Cell Biol. 8 (2007) 722–728.
[66] Y. Xiong, J.D. Uys, K.D. Tew, D.M. Townsend, S-glutathionylation: from molecular mechanisms to health outcomes, Antioxid. Redox Signal. 15 (2011) 233–270.
[67] A. Rokka, V.D. Antonenkov, R. Soininen, H.L. Immonen, P.L. Pirilä, U. Bergmann, R.T. Sormunen, M. Weckström, R. Benz, J.K. Hiltunen, Pxmp2 is a channel-forming protein in mammalian peroxisomal membrane, PLoS One 4 (2009) e5090.
[68] A.J. Meyer, T.P. Dick, Fluorescent protein-based redox probes, Antioxid. Redox Signal. 13 (2010) 621–650.
[69] O. Ivashchenko, P.P. Van Veldhoven, C. Brees, Y.S. Ho, S.R. Terlecky, M. Fransen, Intraperoxisomal redox balance in mammalian cells: oxidative stress and interorganellar cross-talk, Mol. Biol. Cell 22 (2011) 1440–1451.
[70] M. Osiecki, P. Ghanavi, K. Atkinson, L.K. Nielsen, M.R. Doran, The ascorbic acid paradox, Biochem. Biophys. Res. Commun. 400 (2010) 466–470.
[71] K. Croes, V. Foulon, M. Casteels, P.P. Van Veldhoven, G.P. Mannaerts, Phytanoyl-CoA hydroxylase: recognition of 3-methyl-branched acyl-CoAs and requirement for GTP or ATP and Mg(2+) in addition to its known hydroxylation cofactors, J. Lipid Res. 41 (2000) 629–636.
[72] T.B. Dansen, K.W. Wirtz, The peroxisome in oxidative stress, IUBMB Life 51 (2001) 223–230.
[73] A. Broniec, R. Klosinski, A. Pawlak, M. Wrona-Krol, D. Thompson, T. Sarna, Interactions of plasmalogens and their diacyl analogs with singlet oxygen in selected model systems, Free Radic. Biol. Med. 50 (2011) 892–898.
[74] Y. Yano, M. Oku, N. Akeyama, A. Itoyama, H. Yurimoto, S. Kuge, Y. Fujiki, Y. Sakai, A novel fluorescent sensor protein for visualization of redox states in the cytoplasm and in peroxisomes, Mol. Cell. Biol. 30 (2010) 3758–3766.
[75] X. Chen, X. Tian, I. Shin, J. Yoon, Fluorescent and luminescent probes for detection of reactive oxygen and nitrogen species, Chem. Soc. Rev. 40 (2011) 4783–4804.
[76] M.B. Cannon, S.J. Remington, Redox-sensitive green fluorescent protein: probes for dynamic intracellular redox responses. A review, Methods Mol. Biol. 476 (2008) 51–65.
[77] G.T. Hanson, R. Aggeler, D. Oglesbee, M. Cannon, R.A. Capaldi, R.Y. Tsien, S.J. Remington, Investigating mitochondrial redox potential with redox-sensitive green fluorescent protein indicators, J. Biol. Chem. 279 (2004) 13044–13053.
[78] A.J. Meyer, T. Brach, L. Marty, S. Kreye, N. Rouhier, J.P. Jacquot, R. Hell, Redox-sensitive GFP in *Arabidopsis thaliana* is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer, Plant J. 52 (2007) 973–986.
[79] M. Schwarzländer, M.D. Fricker, L.J. Sweetlove, Monitoring the in vivo redox state of plant mitochondria: effect of respiratory inhibitors, abiotic stress and assessment of recovery from oxidative challenge, Biochim. Biophys. Acta 1787 (2009) 468–475.
[80] M. van Lith, S. Tiwari, J. Pediani, G. Milligan, N.J. Bulleid, Real-time monitoring of redox changes in the mammalian endoplasmic reticulum, J. Cell Sci. 124 (2011) 2349–2356.
[81] V.V. Belousov, A.F. Fradkov, K.A. Lukyanov, D.B. Staroverov, K.S. Shakhbazov, A.V. Terskikh, S. Lukyanov, Genetically encoded fluorescent indicator for intracellular hydrogen peroxide, Nat. Methods 3 (2006) 281–286.
[82] W. Gehrmann, M. Elsner, A specific fluorescence probe for hydrogen peroxide detection in peroxisomes, Free Radic. Res. 45 (2011) 501–506.
[83] M. Malinouski, Y. Zhou, V.V. Belousov, D.L. Hatfield, V.N. Gladyshev, Hydrogen peroxide probes directed to different cellular compartments, PLoS One 21 (2011) 6.
[84] M. Elsner, W. Gehrmann, S. Lenzen, Peroxisome-generated hydrogen peroxide as important mediator of lipotoxicity in insulin-producing cells, Diabetes 60 (2011) 200–208.
[85] M. Oku, Y. Sakai, Assessment of physiological redox state with novel FRET protein probes, Antioxid. Redox Signal. 16 (2012) 698–704.
[86] J.K. Reddy, D.L. Azarnoff, C.E. Hignite, Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens, Nature 283 (1980) 397–398.
[87] A.V. Yeldandi, M.S. Rao, J.K. Reddy, Hydrogen peroxide generation in peroxisome proliferator-induced oncogenesis, Mutat. Res. 448 (2000) 159–177.
[88] S. Chu, Q. Huang, K. Alvares, A.V. Yeldandi, M.S. Rao, J.K. Reddy, Transformation of mammalian cells by overexpressing hydrogen peroxide-generating peroxisomal fatty acyl-CoA oxidase, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 7080–7084.
[89] M. Okamoto, J.K. Reddy, R. Oyasu, Tumorigenic conversion of a non-tumorigenic rat urothelial cell line by overexpression of hydrogen peroxide-generating peroxisomal fatty acyl-CoA oxidase, Int. J. Cancer 70 (1997) 716–721.

[90] Y. Li, J.C. Tharappel, S. Cooper, M. Glenn, H.P. Glauert, B.T. Spear, Expression of the hydrogen peroxide-generating enzyme fatty acyl-CoA oxidase activates NF-kappaB, DNA Cell Biol. 19 (2000) 113–120.
[91] W. Gehrmann, M. Elsner, S. Lenzen, Role of metabolically generated reactive oxygen species for lipotoxicity in pancreatic β-cells, Diabetes Obes. Metab. 12 (2010) 149–158.
[92] E. Margoliash, A. Novogrodsky, A. Schejter, Irreversible reaction of 3-amino-1,2,4-triazole and related inhibitors with the protein of catalase, Biochem. J. 74 (1960) 339–350.
[93] J.I. Koepke, C.S. Wood, L.J. Terlecky, P.A. Walton, S.R. Terlecky, Progeric effects of catalase inactivation in human cells, Toxicol. Appl. Pharmacol. 232 (2008) 99–108.
[94] X. Chen, H. Liang, H. Van Remmen, J. Vijg, A. Richardson, Catalase transgenic mice: characterization and sensitivity to oxidative stress, Arch. Biochem. Biophys. 422 (2004) 197–210.
[95] M.W. Epperly, J.A. Melendez, X. Zhang, S. Nie, L. Pearce, J. Peterson, D. Franicola, T. Dixon, B.A. Greenberger, P. Komanduri, H. Wang, J.S. Greenberger, Mitochondrial targeting of a catalase transgene product by plasmid liposomes increases radioresistance in vitro and in vivo, Radiat. Res. 171 (2009) 588–595.
[96] M. Price, S.R. Terlecky, D. Kessel, A role for hydrogen peroxide in the pro-apoptotic effects of photodynamic therapy, Photochem. Photobiol. 85 (2009) 1491–1496.
[97] V. Undyala, S.R. Terlecky, R.S. Vander Heide, Targeted intracellular catalase delivery protects neonatal rat myocytes from hypoxia-reoxygenation and ischemia-reperfusion injury, Cardiovasc. Pathol. 20 (2011) 272–280.
[98] M.E. Bulina, D.M. Chudakov, O.V. Britanova, Y.G. Yanushevich, D.B. Staroverov, T.V. Chepurnykh, E.M. Merzlyak, M.A. Shkrob, S. Lukyanov, K.A. Lukyanov, A genetically encoded photosensitizer, Nat. Biotechnol. 24 (2006) 95–99.
[99] P. Carpentier, S. Violot, L. Blanchoin, D. Bourgeois, Structural basis for the phototoxicity of the fluorescent protein KillerRed, FEBS Lett. 583 (2009) 2839–2842.
[100] S.J. Gould, G.A. Keller, S. Subramani, Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins, J. Cell Biol. 107 (1988) 897–905.
[101] P.J. Halvey, J.M. Hansen, J.M. Johnson, Y.M. Go, A. Samali, D.P. Jones, Selective oxidative stress in cell nuclei by nuclear-targeted D-amino acid oxidase, Antioxid. Redox Signal. 9 (2007) 807–816.
[102] M.B. Toledano, A.G. Planson, A. Delaunay-Moisan, Reining in hydrogen peroxide for safe signaling, Cell 140 (2010) 454–456.
[103] M. Forkink, J.A. Smeitink, R. Brock, P.H. Willems, W.J. Koopman, Detection and manipulation of mitochondrial reactive oxygen species in mammalian cells, Biochim. Biophys. Acta 1797 (2010) 1034–1044.
[104] M. Kikuchi, N. Hatano, S. Yokota, N. Shimozawa, T. Imanaka, H. Taniguchi, Proteomic analysis of rat liver peroxisome: presence of peroxisome-specific isozyme of Lon protease, J. Biol. Chem. 279 (2004) 421–428.
[105] E.B. Aksam, A. Koek, J.A. Kiel, S. Jourdan, M. Veenhuis, I.J. van der Klei, A peroxisomal lon protease and peroxisome degradation by autophagy play key roles in vitality of Hansenula polymorpha cells, Autophagy 3 (2007) 96–105.
[106] F. Guéraud, M. Atalay, N. Bresgen, A. Cipak, P.M. Eckl, L. Huc, I. Jouanin, W. Siems, K. Uchida, Chemistry and biochemistry of lipid peroxidation products, Free Radic. Res. 44 (2010) 1098–1124.
[107] R. Fritz, J. Bol, U. Hebling, S. Angermüller, A. Völkl, H.D. Fahimi, S. Mueller, Compartment-dependent management of hydrogen peroxide by peroxisomes, Free Radic. Biol. Med. 42 (2007) 1119–1129.
[108] N.S. Bryan, K. Bian, F. Murad, Discovery of the nitric oxide signaling pathway and targets for drug development, Front. Biosci. 14 (2009) 1–18.
[109] E.A. Veal, A.M. Day, B.A. Morgan, Hydrogen peroxide sensing and signaling, Mol. Cell 26 (2007) 1–14.
[110] M. Rigoulet, E.D. Yoboue, A. Devin, Mitochondrial ROS generation and its regulation: mechanisms involved in hydrogen peroxide signaling, Antioxid. Redox Signal. 14 (2011) 459–468.
[111] V.P. Patel, C.T. Chu, Nuclear transport, oxidative stress, and neurodegeneration, Int. J. Clin. Exp. Pathol. 4 (2011) 215–229.
[112] L. Baird, A.T. Dinkova-Kostova, The cytoprotective role of the Keap1-Nrf2 pathway, Arch. Toxicol. 85 (2011) 241–272.
[113] M.J. Morgan, Z.G. Liu, Crosstalk of reactive oxygen species and NF-κB signaling, Cell Res. 21 (2011) 103–115.
[114] J.W. Zmijewski, E. Lorne, X. Zhao, Y. Tsuruta, Y. Sha, G. Liu, E. Abraham, Antiinflammatory effects of hydrogen peroxide in neutrophil activation and acute lung injury, Am. J. Respir. Crit. Care Med. 179 (2009) 694–704.
[115] V. Oliveira-Marques, H.S. Marinho, L. Cyrne, F. Antunes, Role of hydrogen peroxide in NF-κB activation: from inducer to modulator, Antioxid. Redox Signal. 11 (2009) 2223–2243.
[116] F. Camões, N.A. Bonekamp, H.K. Delille, M. Schrader, Organelle dynamics and dysfunction: a closer link between peroxisomes and mitochondria, J. Inherit. Metab. Dis. 32 (2009) 163–180.
[117] E. Dixit, S. Boulant, Y. Zhang, A.S. Lee, C. Odendall, B. Shum, N. Hacohen, Z.J. Chen, S.P. Whelan, M. Fransen, M.L. Nibert, G. Superti-Furga, J.C. Kagan, Peroxisomes are signaling platforms for antiviral innate immunity, Cell 141 (2010) 668–681.
[118] J.I. Koepke, K.A. Nakrieko, C.S. Wood, K.K. Boucher, L.J. Terlecky, P.A. Walton, S.R. Terlecky, Restoration of peroxisomal catalase import in a model of human cellular aging, Traffic 8 (2007) 1590–1600.
[119] P.P. Van Veldhoven, Biochemistry and genetics of inherited disorders of peroxisomal fatty acid metabolism, J. Lipid Res. 51 (2010) 2863–2895.
[120] Z. Liu, R.A. Butow, Mitochondrial retrograde signaling, Annu. Rev. Genet. 40 (2006) 159–185.
[121] N. Makino, Y. Mochizuki, S. Bannai, Y. Sugita, Kinetic studies on the removal of extracellular hydrogen peroxide by cultured fibroblasts, J. Biol. Chem. 269 (1994) 1020–1025.
[122] C.S. Wood, J.I. Koepke, H. Teng, K.K. Boucher, S. Katz, P. Chang, L.J. Terlecky, I. Papanayotou, P.A. Walton, S.R. Terlecky, Hypocatalasemic fibroblasts accumulate hydrogen peroxide and display age-associated pathologies, Traffic 7 (2006) 97–107.
[123] B. Xu, J.T. Moritz, P.N. Epstein, Overexpression of catalase provides partial protection to transgenic mouse beta cells, Free Radic. Biol. Med. 27 (1999) 830–837.
[124] S. Mueller, A. Weber, R. Fritz, S. Mütze, D. Rost, H. Walczak, A. Völkl, W. Stremmel, Sensitive and real-time determination of hydrogen peroxide release from intact peroxisomes, Biochem. J. 363 (2002) 483–491.
[125] M. Muller, Cellular senescence: molecular mechanisms, in vivo significance, and redox considerations, Antioxid. Redox Signal. 11 (2009) 59–98.
[126] F.G. Sheikh, K. Pahan, M. Khan, E. Barbosa, I. Singh, Abnormality in catalase import into peroxisomes leads to severe neurological disorder, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 2961–2966.
[127] A.F. Carvalho, M.P. Pinto, C.P. Grou, I.S. Alencastre, M. Fransen, C. Sá-Miranda, J.E. Azevedo, Ubiquitination of mammalian Pex5p, the peroxisomal import receptor, J. Biol. Chem. 282 (2007) 31267–31272.
[128] C. Klomsiri, P.A. Karplus, L.B. Poole, Cysteine-based redox switches in enzymes, Antioxid. Redox Signal. 14 (2011) 1065–1077.
[129] P.K. Kim, D.W. Hailey, R.T. Mullen, J. Lippincott-Schwartz, Ubiquitin signals autophagic degradation of cytosolic proteins and peroxisomes, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 20567–20574.
[130] S. Hara-Kuge, Y. Fujiki, The peroxin Pex14p is involved in LC3-dependent degradation of mammalian peroxisomes, Exp. Cell Res. 314 (2008) 3531–3541.
[131] S.J. Huybrechts, P.P. Van Veldhoven, C. Brees, G.P. Mannaerts, G.V. Los, M. Fransen, Peroxisome dynamics in cultured mammalian cells, Traffic 10 (2009) 1722–1733.
[132] V.M. Hubbard, R. Valdor, F. Macian, A.M. Cuervo, Selective autophagy in the maintenance of cellular homeostasis in aging organisms, Biogerontology 13 (2012) 21–35.
[133] Y.M. Hosakote, P.D. Jantzi, D.L. Esham, H. Spratt, A. Kurosky, A. Casola, R.P. Garofalo, Viral-mediated inhibition of antioxidant enzymes contributes to the pathogenesis of severe respiratory syncytial virus bronchiolitis, Am. J. Respir. Crit. Care Med. 183 (2011) 1550–1560.
[134] M. Baes, P. Aubourg, Peroxisomes, myelination, and axonal integrity in the CNS, Neuroscientist 15 (2009) 367–379.
[135] S. Ferdinandusse, A.W. Zomer, J.C. Komen, C.E. van den Brink, M. Thanos, F.P. Hamers, R.J. Wanders, P.T. van der Saag, B.T. Poll-The, P. Brites, Ataxia with loss of Purkinje cells in a mouse model for Refsum disease, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 17712–17717.
[136] A. Pujol, C. Hindelang, N. Callizot, U. Bartsch, M. Schachner, J.L. Mandel, Late onset neurological phenotype of the X-ALD gene inactivation in mice: a mouse model for adrenomyeloneuropathy, Hum. Mol. Genet. 11 (2002) 499–505.
[137] M. Baes, P. Gressens, E. Baumgart, P. Carmeliet, M. Casteels, M. Fransen, P. Evrard, D. Fahimi, P.E. Declercq, D. Collen, P.P. Van Veldhoven, G.P. Mannaerts, A mouse model for Zellweger syndrome, Nat. Genet. 17 (1997) 49–57.
[138] C.M. Kassmann, C. Lappe-Siefke, M. Baes, B. Brügger, A. Mildner, H.B. Werner, O. Natt, T. Michaelis, M. Prinz, J. Frahm, K.A. Nave, Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes, Nat. Genet. 39 (2007) 969–976.
[139] L. Hulshagen, O. Krysko, A. Bottelbergs, S. Huyghe, R. Klein, P.P. Van Veldhoven, P.P. De Deyn, R. D'Hooge, D. Hartmann, M. Baes, Absence of functional peroxisomes from mouse CNS causes dysmyelination and axon degeneration, J. Neurosci. 28 (2008) 4015–4027.
[140] J. López-Erauskin, S. Fourcade, J. Galino, M. Ruiz, A. Schlüter, A. Naudi, M. Jove, M. Portero-Otin, R. Pamplona, I. Ferrer, A. Pujol, Antioxidants halt axonal degeneration in a mouse model of X-adrenoleukodystrophy, Ann. Neurol. 70 (2011) 84–92.
[141] C.M. Kassmann, S. Quintes, J. Rietdorf, W. Möbius, M.W. Sereda, T. Nientiedt, G. Saher, M. Baes, K.A. Nave, A role for myelin-associated peroxisomes in maintaining paranodal loops and axonal integrity, FEBS Lett. 585 (2011) 2205–2211.
[142] E. Baumgart, I. Vanhorebeek, M. Grabenbauer, M. Borgers, P.E. Declercq, H.D. Fahimi, M. Baes, Mitochondrial alterations caused by defective peroxisomal biogenesis in a mouse model for Zellweger syndrome (PEX5 knockout mouse), Am. J. Pathol. 159 (2001) 1477–1494.
[143] J. Hirrlinger, A. Resch, J.M. Gutterer, R. Dringen, Oligodendroglial cells in culture effectively dispose of exogenous hydrogen peroxide: comparison with cultured neurones, astroglial and microglial cells, J. Neurochem. 82 (2002) 635–644.
[144] V. Poitout, R.P. Robertson, Minireview: Secondary beta-cell failure in type 2 diabetes — a convergence of glucotoxicity and lipotoxicity, Endocrinology 143 (2002) 339–342.
[145] H. Zhang, K. Ollinger, U. Brunk, Insulinoma cells in culture show pronounced sensitivity to alloxan-induced oxidative stress, Diabetologia 38 (1995) 635–641.
[146] S. Lenzen, J. Drinkgern, M. Tiedge, Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues, Free Radic. Biol. Med. 20 (1996) 463–466.
[147] L. Góth, P. Rass, A. Páy, Catalase enzyme mutations and their association with diseases, Mol. Diagn. 8 (2004) 141–149.
[148] H.M. Brown-Borg, S.G. Rakoczy, Catalase expression in delayed and premature aging mouse models, Exp. Gerontol. 35 (2000) 199–212.

[149] S.E. Schriner, N.J. Linford, G.M. Martin, P. Treuting, C.E. Ogburn, M. Emond, P.E. Coskun, W. Ladiges, N. Wolf, H. Van Remmen, D.C. Wallace, P.S. Rabinovitch, Extension of murine life span by overexpression of catalase targeted to mitochondria, Science 308 (2005) 1909–1911.
[150] A.Y. Seo, A.M. Joseph, D. Dutta, J.C. Hwang, A.P. Aris, C. Leeuwenburgh, New insights into the role of mitochondria in aging: mitochondrial dynamics and more, J. Cell Sci. 123 (2010) 2533–2542.
[151] S.R. Pyper, N. Viswakarma, S. Yu, J.K. Reddy, PPARalpha: energy combustion, hypolipidemia, inflammation and cancer, Nucl. Recept. Signal. 16 (2010) 8:e002.
[152] K. Ishii, L.X. Zhen, D.H. Wang, Y. Funamori, K. Ogawa, K. Taketa, Prevention of mammary tumorigenesis in acatalasemic mice by vitamin E supplementation, Jpn. J. Cancer Res. 87 (1996) 680–684.
[153] K. Yamada, A. Ito, H. Watanabe, T. Takahashi, N.H. Basaran, T. Gotoh, High sensitivity to hepato-tumorigenesis in hypocatalasemic C3H/C(s)b/Gen mice exposed to low doses of 252Cf fission neutrons and 60Co gamma-rays, Anticancer Res. 17 (1997) 2041–2047.
[154] C. Lauer, A. Völkl, S. Riedl, H.D. Fahimi, K. Beier, Impairment of peroxisomal biogenesis in human colon carcinoma, Carcinogenesis 20 (1999) 985–989.
[155] J.A. Litwin, K. Beier, A. Völkl, W.J. Hofmann, H.D. Fahimi, Immunocytochemical investigation of catalase and peroxisomal lipid beta-oxidation enzymes in human hepatocellulartumors and liver cirrhosis, Virchows Arch. 435 (1999) 486–495.
[156] W.M. Frederiks, K.S. Bosch, K.A. Hoeben, J. van Marle, S. Langbein, Renal cell carcinoma and oxidative stress: the lack of peroxisomes, Acta Histochem. 112 (2010) 364–371.
[157] Y. Yang, Y. Song, J. Loscalzo, Regulation of the protein disulfide proteome by mitochondria in mammalian cells, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 10813–10817.
[158] X. Liu, A. Samouilov, J.R. Lancaster, J.L. Zweier, Nitric oxide uptake by erythrocytes is primarily limited by extracellular diffusion not membrane resistance, J. Biol. Chem. 277 (2002) 26194–26199.
[159] G.D. Mao, M.J. Poznansky, Electron spin resonance study on the permeability of superoxide radicals in lipid bilayers and biological membranes, FEBS Lett. 305 (1992) 233–236.
[160] G.P. Bienert, J.K. Schjoerring, T.P. Jahn, Membrane transport of hydrogen peroxide, Biochim. Biophys. Acta 1758 (2006) 994–1003.