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Peroxisomes sense and respond to environmental cues by regulating ROS and RNS signalling networks

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- **Background** Peroxisomes are highly dynamic, metabolically active organelles that used to be regarded as a sink for H₂O₂ generated in different organelles. However, peroxisomes are now considered to have a more complex function, containing different metabolic pathways, and they are an important source of reactive oxygen species (ROS), nitric oxide (NO) and reactive nitrogen species (RNS). Over-accumulation of ROS and RNS can give rise oxidative and nitrosative stress, but when produced at low concentrations they can act as signalling molecules.
- **Scope** This review focuses on the production of ROS and RNS in peroxisomes and their regulation by antioxidants. ROS production is associated with metabolic pathways such as photorespiration and fatty acid β -oxidation, and disturbances in any of these processes can be perceived by the cell as an alarm that triggers defence responses. Genetic and pharmacological studies have shown that photorespiratory H₂O₂ can affect nuclear gene expression, regulating the response to pathogen infection and light intensity. Proteomic studies have shown that peroxisomal proteins are targets for oxidative modification, S-nitrosylation and nitration and have highlighted the importance of these modifications in regulating peroxisomal metabolism and signalling networks. The morphology, size, number and speed of movement of peroxisomes can also change in response to oxidative stress, meaning that an ROS/redox receptor is required. Information available on the production and detection of NO/RNS in peroxisomes is more limited. Peroxisomal homeostasis is critical for maintaining the cellular redox balance and is regulated by ROS, peroxisomal proteases and autophagic processes.
- **Conclusions** Peroxisomes play a key role in many aspects of plant development and acclimation to stress conditions. These organelles can sense ROS/redox changes in the cell and thus trigger rapid and specific responses to environmental cues involving changes in peroxisomal dynamics as well as ROS- and NO-dependent signalling networks, although the mechanisms involved have not yet been established. Peroxisomes can therefore be regarded as a highly important decision-making platform in the cell, where ROS and RNS play a determining role.

Key words: Antioxidants, arabidopsis, autophagy, β -oxidation, nitric oxide, nitrosative stress, oxidative stress, photorespiration, peroxisomes, reactive nitrogen species, RNS, reactive oxygen species, ROS, signalling.

INTRODUCTION

Peroxisomes are ubiquitous organelles in eukaryotic cells and have a close relationship with chloroplasts and mitochondria (Fig. 1). Initially, these organelles were regarded as involved in removing H₂O₂ produced by different sources both inside and outside peroxisomes by catalases. The role of peroxisomes was therefore limited to cleaning up reactive oxygen species (ROS) produced by other organelles. However, in recent years biochemical, transcriptomic and proteomic approaches have demonstrated that these organelles are much more complex and perform functions hitherto unknown (Reumann *et al.*, 2009; Hu *et al.*, 2012; Sandalio *et al.*, 2013). Peroxisomes are highly dynamic, metabolically active organelles that participate in different cellular processes involved in development, morphogenesis and cell responses to stress (del Río *et al.*, 1998; Sandalio *et al.*, 2013). Changes in metabolic pathways, number, size, morphology and even speed of movement in peroxisomes can take place depending on cell type, tissue, organism, developmental stage (Hu *et al.*, 2012) or environmental conditions involving herbicides, clofibrate, metals, wounding or nutrient disturbances

among others (Romero-Puertas *et al.*, 2004; Rodríguez-Serrano *et al.*, 2009; Sinclair *et al.*, 2009; Hu *et al.*, 2012; McCarthy-Suarez *et al.*, 2011; Sandalio *et al.*, 2013) and in response to pathogens (Koh *et al.*, 2005; Kuzniak and Skłodowska, 2005). Photomorphogenesis and senescence are also associated with changes in the metabolism and dynamics of peroxisomes (del Río *et al.*, 1998; Rosenwasser *et al.*, 2011; Hu *et al.*, 2012). In addition, peroxisomes are an important source of ROS and reactive nitrogen species (RNS) and contain a complex battery of antioxidant defences that regulate the accumulation of ROS and RNS. Overproduction of ROS and RNS can cause severe oxidative and nitrosative damage, respectively (del Río, 2011). However, ROS and RNS can also act as signalling molecules that regulate developmental processes and stress responses (Neill *et al.*, 2008; Mittler *et al.*, 2011).

Peroxisomes can also play an important role in cellular redox homeostasis, which is, in turn, a key element in the regulation of cell metabolic pathways (Foyer and Noctor, 2003; Yun *et al.*, 2012). It has been demonstrated that peroxisomes can

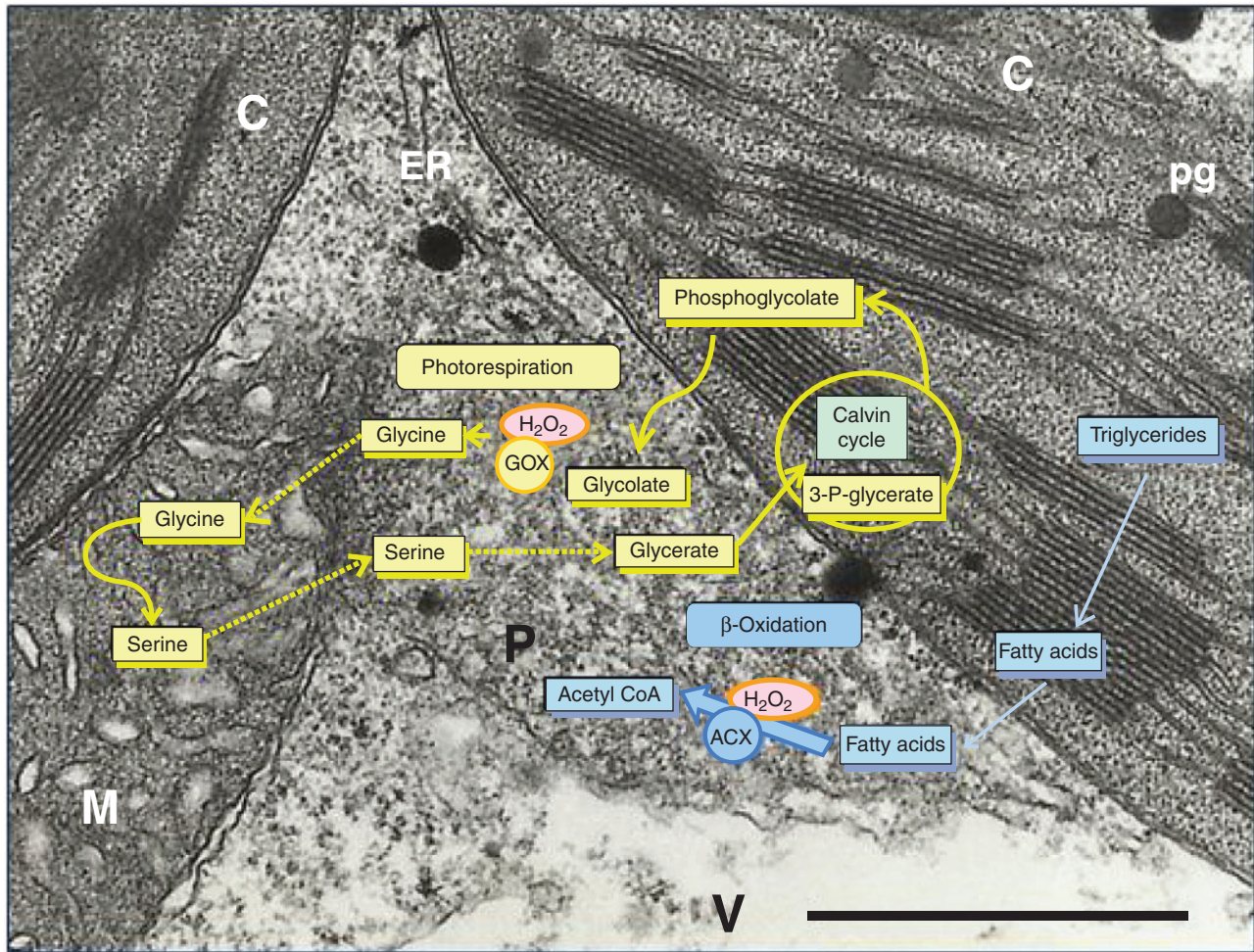


Fig. 1. Electron micrograph of Arabidopsis leaves showing a close relationship between chloroplasts, mitochondria and peroxisomes. Schemes showing H_2O_2 production in the reaction of glycolate oxidase (GOX) during photorespiration involving chloroplasts, peroxisomes and mitochondria (yellow) and H_2O_2 production associated with acyl CoA oxidase (ACX) activity from fatty acid β -oxidation (blue; chloroplasts and peroxisomes). C, chloroplast; ER, endoplasmic reticulum; M, mitochondria; P, peroxisome; pg, plastoglobuli; V, vacuole. Scale bar = 1 μ m.

rapidly sense changes in their environment and modify their metabolism and dynamics, all of which is regulated by ROS (Rodríguez-Serrano *et al.*, 2009; Sinclair *et al.*, 2009; Hu *et al.*, 2012). However, the mechanisms involved in the perception of these stimuli have not yet been established. This review focuses on the production of ROS and RNS in peroxisomes and their regulation by antioxidants, and attempts to connect up all the pieces of the puzzle available in order to determine how peroxisomes perceive changes in their environment and trigger cell responses using ROS and RNS as key molecules.

PEROXISOMES ARE AN IMPORTANT SOURCE OF ROS AND RNS

Peroxisomes produce and scavenge different ROS

The term ROS includes oxygen-derivative species obtained through the reduction of oxygen, such as superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals ($\cdot OH$), alkoxyl radicals ($RO\cdot$) and peroxy radicals ($ROO\cdot$), and also some non-radical compounds, such as H_2O_2 , the excited oxygen species singlet oxygen (1O_2),

ozone (O_3) and hypochlorous acid ($HOCl$) (Halliwell and Gutteridge, 2007). The reactivity of each species differs, although $\cdot OH$ is considered to be the strongest oxidant, capable of reacting with all types of molecule (Halliwell and Gutteridge, 2007). Different sources of ROS, including H_2O_2 , $O_2^{\cdot-}$ and $\cdot OH$, have been reported to be produced in peroxisomes (Fig. 2). Superoxide radical production takes place in a short electron chain associated with the NADH/NADPH-driven peroxisomal membrane and also in the peroxisomal matrix associated with xanthine oxidoreductase (XOD/XDH) and uricase, both of which are important enzymes in the catabolism of nucleic acids and ureide metabolism, respectively (Sandalo *et al.*, 2013) (Fig. 2). Recently, sulphite oxidase has been added to the list of peroxisomal enzymes involved in $O_2^{\cdot-}$ production associated with sulphite detoxification and assimilatory reduction of sulphate. However, the function of this enzyme in peroxisomes has not been clearly established and may prevent oxidative damage to sulphite (Byrne *et al.*, 2009) (Fig. 2). In animal tissues it has been suggested that nitric oxide synthase (NOS) present in these organelles can also produce significant amounts of $O_2^{\cdot-}$ in the absence of an adequate amount of its

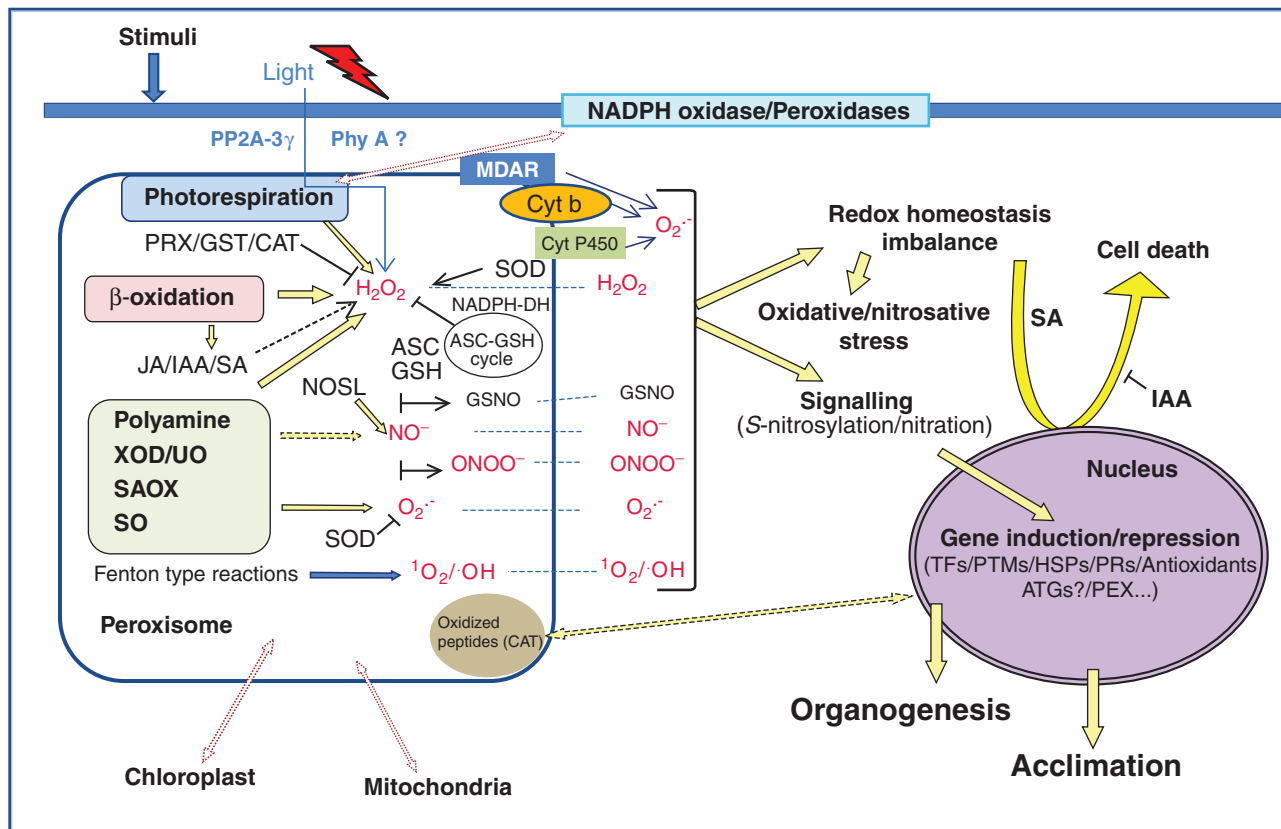


Fig. 2. Schematic overview of the signalling networks involving ROS and RNS in peroxisomes. H_2O_2 and $\text{O}_2^{\cdot-}$ are produced in different metabolic pathways and in a small electron transport chain associated with the membrane and their steady state is regulated by SODs, catalase, the ASC–GSH cycle and probably peroxiredoxin (PRX). ROS, particularly H_2O_2 , can diffuse to the cytosol where they can alter redox homeostasis and regulate gene transcription in the nucleus, giving rise to acclimatization or cell death depending on the stimuli. Light can regulate photorespiration peroxisome-dependent changes in cytosolic redox homeostasis that cause SA-dependent lesions and induction of pathogenesis-related proteins. This process is dependent on SA and can also be regulated by PP2A-B'γ and Phy A. In addition, peroxisomes are a source of hormones, such as JA and IAA, that participate in the network regulating cell responses to stress (i.e. IAA, which negatively regulates light-dependent cell death). NO is produced in peroxisomes by a NOS-like activity, although other sources can also be involved, such as xanthine oxidase (XOD) and polyamines. ONOO⁻ and GSNO are produced in peroxisomes by reaction of NO with $\text{O}_2^{\cdot-}$ and GSH, respectively, and can also act as signal molecules regulating gene expression and protein activity throughout post-translational modifications (PTMs) such as nitration and S-nitrosylation. Oxidized peptides may also regulate gene expression and autophagy in order to control homeostasis and peroxisomal quality. Peroxisomal ROS promote cross-talk with mitochondria and chloroplasts, probably through changes in the redox state of each organelle. ATGs, autophagy-related genes; CAT, catalase; Cyt P450, cytochrome P450 reductase; GST, glutathione-S-transferase; HSPs, heat shock proteins; IAA, indoleacetic acid; JA, jasmonic acid; MDAR, monodehydroascorbate reductase; NADPH-DH, NADPH dehydrogenases; NOS-L, NO synthase-like activity; ONOO⁻, peroxynitrite; PRX, peroxiredoxin; PEX, peroxins; Phy A, phytochrome A; PP2A-B'γ, 2A protein phosphatase subunit; PRs, pathogenesis-related proteins; SAOX, sarcosine oxidase; SO, sulphite oxidase; TFs, transcription factors; UO, urate oxidase.

substrate (Stuehr *et al.*, 2001). However, a gene orthologous to the mammalian gene for NOS, whose identity remains elusive, has not been found in plants (Moreau *et al.*, 2010).

The production of H_2O_2 in peroxisomes takes place in association with different metabolic pathways. Photorespiration is a light-dependent process that results in the uptake of O_2 and the release of CO_2 and is compartmentalized in chloroplasts, peroxisomes and mitochondria (Foyer *et al.*, 2010) (Fig. 1). Glycolate oxidase (GOX) is one of the key enzymes in the photorespiration cycle and is probably the main source of H_2O_2 in green tissues (Fig. 1). The rate of H_2O_2 production through this process is 2-fold higher than in chloroplasts and 50-fold higher than in mitochondria (Foyer and Noctor, 2003). Fatty acid β -oxidation is another important source of H_2O_2 in peroxisomes (Fig. 1). This metabolic pathway is more active in germinating seeds, while being ubiquitous in green tissues and also involved in the metabolism of phytohormones such as jasmonic acid (JA) and auxins (Hooks, 2002). Other functions associated with

β -oxidation have recently been described in non-storage tissue, such as benzoic acid, biotin and isoprenoid biosynthesis (Hu *et al.*, 2012; Linka and Theodoulou, 2013; Cassin-Ross and Hu, 2014). Acyl CoA oxidase (ACX) is the principal enzyme in this pathway and catalyses the oxidation of acyl-CoA to trans-2-enoyl-CoA to produce H_2O_2 as a by-product (Kaur *et al.*, 2009). The spontaneous or enzymatic dismutation of $\text{O}_2^{\cdot-}$ catalysed by superoxide dismutases (SODs) is another source of H_2O_2 . The presence of Mn-SOD and CuZn-SOD in peroxisomes has been demonstrated in different plant species (Sandalio *et al.*, 2013) (Fig. 2). Sarcosine oxidase has been added to the list of candidates for peroxisomal H_2O_2 sources in plants and catalyses the oxidation of sarcosine, N-methyl amino acids and l-pipecolate, producing formaldehyde, glycine and H_2O_2 (Goyer *et al.*, 2004) (Fig. 2). The catabolism of polyamines is further source of H_2O_2 in peroxisomes, where diamine oxidases and polyamine oxidases have been identified (Kamada-Nobusada *et al.*, 2008; Osno *et al.*, 2012; Planas-Portell *et al.*, 2013). The presence of

haem- and non-haem iron-containing proteins and ascorbate in peroxisomes may contribute to $\cdot\text{OH}$ production in these organelles through Fenton-type reactions. In peroxisomes purified from watermelon cotyledons, the production of $\cdot\text{OH}$ was demonstrated by electron spin resonance spectroscopy (Sandalio *et al.*, 1988). Fenton-type reactions may also give rise to the production in peroxisomes of singlet oxygen, an ROS typically associated with the chloroplast, as it has been reported by using the fluorescent probe Singlet Oxygen Sensor Green (SOSG; Mor *et al.*, 2014). Thus, $^1\text{O}_2$ could be a new player in the peroxisome-derived signalling network.

The steady-state levels of ROS in peroxisomes are strongly regulated by scavenging systems, including enzymatic and non-enzymatic antioxidants. Plant peroxisomes contain enzymatic defences to cope with different types of ROS (Table 1). Superoxide dismutases are involved in removing superoxide radicals, with the presence of two isoforms of SOD containing Cu and Zn (CuZn-SOD) and Mn (Mn-SOD) being reported in peroxisomes from different plant species (Sandalio *et al.*, 2013) and a Fe-SOD in carnation petals (Droillard and Paulin, 1990). Catalase is the main antioxidant involved in removing H_2O_2 (Huang *et al.*, 1983; Mhamdi *et al.*, 2012). The ascorbate–glutathione cycle, made up of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), ascorbate (ASC) and glutathione (GSH) (Jiménez *et al.*, 1997; Romero-Puertas *et al.*, 2006; Mhamdi *et al.*, 2012), is also involved in maintaining H_2O_2 levels under control in peroxisomes. NADPH, which is necessary for the ASC–GSH cycle, is supplied by isocitrate dehydrogenase (ICDH) and the pentose phosphate cycle present in these organelles (Corpas *et al.*, 1999; Reumann *et al.*, 2009). Peroxisomes can also produce NADPH *de novo* through NADH phosphorylation (Waller *et al.*, 2010). Other peroxidases may be present in peroxisomes, such as glutathione peroxidase and peroxiredoxins (Dietz, 2003), although neither the proteins nor the genes have yet been characterized. To our knowledge, the presence of thioredoxins in peroxisomes has not yet been demonstrated, although the identification of uricase as a target of thioredoxins suggests the presence of thioredoxins in peroxisomes (Du *et al.*, 2010). The presence of glutathione transferases in peroxisomes from arabidopsis plants has also been reported (Dixon *et al.*, 2009). These enzymes require GSH and participate in the detoxification of xenobiotic compounds and lipid peroxide products in addition to removing H_2O_2 (Dixon *et al.*, 2009).

Oxidative stress can lead directly to reversible or irreversible protein modifications. Protein carbonylation is a highly important oxidative-derived modification that consists of carbonyl radical production and is considered to be an irreversible process. The increase in carbonylated proteins in peroxisomes has been observed under different stress conditions associated with an increase in H_2O_2 , such as cadmium toxicity (Romero-Puertas *et al.*, 2002), senescence (Vanaker *et al.*, 2006), treatment with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D; McCarthy *et al.*, 2011), combined salt and continuous light treatment (Mano *et al.*, 2014) and, in peroxisomes, treatment with metals to induce Fenton-derived $\cdot\text{OH}$ production (Nguyen and Donaldson, 2005). The proteome of carbonylated proteins in peroxisomes has been studied in different plant species, and several proteins prone to carbonylation have been identified,

some of which participate in antioxidant defences, fatty acid β -oxidation or cell death (Table 2) (Romero-Puertas *et al.*, 2002; Nguyen and Donaldson, 2005; Mano *et al.*, 2014). Oxidized proteins are dangerous and need to be proteolytically degraded by proteases to preserve peroxisomal functionality. The presence of serine protease activity has been demonstrated in purified peroxisomes from pea leaves using biochemical techniques, with catalase, GOX and glucose 6-P dehydrogenase (G6PDH) being the target of these proteases (Distefano *et al.*, 1999; Romero-Puertas *et al.*, 2002). A cysteine protease, resistant to drought 21A-like 1 (RDL1), has also been shown to be involved in fatty acid β -oxidation and development, although its substrate is still unknown (Quan *et al.*, 2013). However, by using a genetic technique, damaged or obsolete proteins have been demonstrated to be transported outside the organelles for degradation by LON2 proteases with the assistance of the peroxins PEX4, PEX6 and PEX22, involved in protein import into peroxisomes (Farmer *et al.*, 2013). In peroxisomes from yeast, fungi and mammal cells, LON proteases have been shown to be capable of selectively degrading oxidatively damaged peroxisomal matrix proteins and may act as a key player in the coordination of ROS metabolism in peroxisomes (Kumar *et al.*, 2014; Nordgren and Fransen, 2014). A genetic screen for suppressors of the *lon2* mutant has demonstrated that the degradation of proteins from the glyoxylate cycle during the functional transition of glyoxysomes to leaf peroxisomes, requiring not only LON2 but also autophagy, is involved in this process (Farmer *et al.*, 2013; Shibata *et al.*, 2014).

Peroxisomes produce NO and RNS

In addition to ROS, NO and its derivative RNS can also be produced in peroxisomes. Nitric oxide is a gaseous free radical produced in almost all eukaryotic cells and has attracted considerable attention in the last decade (Yun *et al.*, 2012; Chen *et al.*, 2014). Although NO has emerged as an important plant signalling molecule, our current knowledge of the metabolic sources of NO and the mechanisms involved in NO scavenging in plants is incomplete (Mur *et al.*, 2013). Most NO produced in animal systems is due to the enzyme NOS, and NOS2, the inducible form of this enzyme, displays a dual cytosolic–peroxisomal localization in hepatocytes (Stolz *et al.*, 2002; Pacher *et al.*, 2007). However, in plants, NO can be produced by both enzymatic and non-enzymatic sources (Gupta *et al.*, 2011). A gene orthologous to NOS from animals has not been found in plants, although several attempts to identify plant NOS have been described. However, the data were not reproducible at a later stage or the protein was not directly involved in NO production (Moreau *et al.*, 2010). Several studies, mostly based on the use of NOS inhibitors, have suggested the presence of an NOS-like activity in plants, and the presence of this activity has also been described in peroxisomes (del Río, 2011). Additionally, peroxisomes contain xanthine oxidase (XOD), which is also capable of generating NO (Godber *et al.*, 2000; Antonenkov *et al.*, 2010). Polyamine catabolism has been identified as another source of NO (Wimalasekera *et al.*, 2011) in plants and, because peroxisomes contain polyamine oxidases and amine oxidases, it is possible to speculate that polyamines may contribute to NO production in peroxisomes.

TABLE 1. Enzymatic antioxidant defences present in plant peroxisomes

Antioxidant	Substrate	References
Mn-superoxide dismutase	O_2^-	Droillard and Paulin, 1990; Rodríguez-Serrano <i>et al.</i> , 2007;
CuZn-superoxide dismutase		Sandalo <i>et al.</i> , 2013
Fe-superoxide dismutase		
Catalase	H_2O_2	Huang <i>et al.</i> , 1983; Mhamdi <i>et al.</i> , 2012
Ascorbate peroxidase	H_2O_2 , organic hydroperoxides	Jiménez <i>et al.</i> , 1997; Lisenbee <i>et al.</i> , 2003; Leterrier <i>et al.</i> , 2005
Glutathione reductase	GSSG	Romero-Puertas <i>et al.</i> , 2006
Monodehydroascorbate reductase	Monodehydroascorbate	Jiménez <i>et al.</i> , 1997; Lisenbee <i>et al.</i> , 2005
Dehydroascorbate reductase	Dehydroascorbate	Jiménez <i>et al.</i> , 1997; Reumann <i>et al.</i> , 2009
Peroxiredoxin	H_2O_2 , organic hydroperoxides, ONOO ⁻	Verdoucq <i>et al.</i> , 1999; Dietz, 2003
Glutathione S-transferase	H_2O_2 , organic hydroperoxides	Dixon <i>et al.</i> , 2009
NADPH-generating dehydrogenases	NADP	Corpas <i>et al.</i> , 1999; Reumann <i>et al.</i> , 2009;
Isocitrate dehydrogenase		Mhamdi and Noctor, 2015
Glucose 6-phosphate dehydrogenase		
6-Phosphogluconate dehydrogenase		

TABLE 2. Main peroxisomal proteins identified as target of post-translational modifications associated with ROS and RNS

Protein	Carbonylation	S-nitrosylation	Nitration
Catalase	Romero-Puertas <i>et al.</i> , 2002	Ortega-Galisteo <i>et al.</i> , 2012	
Glutathione reductase	Romero-Puertas <i>et al.</i> , 2002		
Mn-superoxide dismutase	Romero-Puertas <i>et al.</i> , 2002		
Glycolate oxidase		Ortega-Galisteo <i>et al.</i> , 2012	Lozano-Juste <i>et al.</i> , 2011
Hydroxypyruvate reductase		Ortega-Galisteo <i>et al.</i> , 2012	Corpas <i>et al.</i> , 2013
Malate synthase	Nguyen and Donaldson, 2005		
Isocitrate lyase	Nguyen and Donaldson, 2005		
Malate dehydrogenase	Nguyen and Donaldson, 2005	Ortega-Galisteo <i>et al.</i> , 2012	Lozano-Juste <i>et al.</i> , 2011
Serine-glyoxylate aminotransferase		Ortega-Galisteo <i>et al.</i> , 2012	
Aminotransferase 1		Ortega-Galisteo <i>et al.</i> , 2012	
Nitrile-specifier protein 5 nitrilase 1	Mano <i>et al.</i> , 2014		
Nucleoside diphosphate kinase AIM1 protein	Mano <i>et al.</i> , 2014		
Glycine-rich RNA-binding protein 8	Mano <i>et al.</i> , 2014		
Aldehyde dehydrogenase family 7 member B4	Mano <i>et al.</i> , 2014		

The presence of NO in plant peroxisomes under physiological and stress conditions has been described (Corpas *et al.*, 2004; del Río, 2011). Nitric oxide promotes the formation of peroxynitrite (ONOO⁻) and nitrosoglutathione (GSNO) in the presence of O_2^- and GSH, respectively. Peroxynitrite has recently been detected in peroxisomes from arabidopsis (Corpas and Barroso, 2014), and some peroxisomal proteins, such as GOX, malate dehydrogenase (MDH) and hydroxypyruvate reductase (HPR) (Lozano-Juste *et al.*, 2011; Corpas *et al.*, 2013) (Table 2), have been previously described as targets of nitration. Nitrosoglutathione, considered to be a cellular reservoir of NO bioactivity, has been localized in pea leaf peroxisomes by electron microscopic immunocytochemistry (Ortega-Galisteo *et al.*, 2012). A proteomic study has shown that six peroxisomal proteins are targets of S-nitrosylation involved in β -oxidation (MDH), photorespiration (HPR, GOX, serine-glyoxylate aminotransferase and aminotransferase 1) and the antioxidant system (catalase) (Ortega-Galisteo *et al.*, 2012) (Table 2).

RNS are important signalling molecules that influence many aspects of cell function, including differentiation and cell survival in response to environmental cues. An increase in peroxisomal NO and RNS production has been shown under

different stress conditions, such as salinity and cadmium stress (Corpas *et al.*, 2009; Corpas and Barroso, 2014). On the other hand, NO can re-establish catalase activity, which was observed to decrease in cadmium-treated sunflowers plants (Laspina *et al.*, 2005) and to negatively affect the catalase capacity of cadmium-treated cells (De Michele *et al.*, 2009). Additionally, the S-nitrosylation levels of catalase and glycolate oxidase changed under abiotic stresses from cadmium and 2,4-D, suggesting that this modification may be involved in regulating peroxisomal H_2O_2 levels under physiological and stress conditions (Ortega-Galisteo *et al.*, 2012).

Peroxisomal NO has emerged as a new player in auxin-induced root organogenesis. The spatially and temporally coordinated release of NO and indoleacetic acid (IAA) from peroxisomes has been demonstrated to promote lateral root formation induced by indole-3-butyric acid (Schlicht *et al.*, 2013). It has also been shown that pollen tube growth and re-orientation in *Lilium longiflorum* are regulated by NO production in peroxisomes (Prado *et al.*, 2008). Peroxisomal NO may also participate in epinasty development induced by the herbicide 2,4-D through the post-transductional modification of actin (Pazmiño *et al.*, 2014; Rodríguez-Serrano *et al.*, 2014). Additionally, NO has been reported to be both a pro-oxidant

and an antioxidant in plants, and NO pre-incubation has been shown to delay loss of catalase activity in barley aleurone cells (Beligni *et al.*, 2002).

Some RNS are highly unstable and can cause direct oxidative and nitrosative damage, such as lipid peroxidation (Yin *et al.*, 2011). In this context, the presence in peroxisomes of antioxidants capable of regulating RNS, such as SOD, that can remove superoxide and thus avoid ONOO⁻ formation is essential. Although the presence of peroxiredoxins or thioredoxins in peroxisomes, which may regulate peroxynitrite and S-nitrosothiol levels, is predictable, it still requires experimental confirmation. It is also important to mention that the regulation of NO and RNS levels is an issue that needs further study in plant cells peroxisome.

PEROXISOMES PLAY A KEY ROLE IN MAINTAINING CELLULAR REDOX HOMEOSTASIS

Peroxisomes contain many enzymes involved in producing and degrading ROS/RNS and therefore may modulate the cellular redox balance. Glutathione and ascorbic acid are major non-enzymatic cellular redox buffer systems, with S-nitrosylation emerging as a key redox-based post-translational modification (Yun *et al.*, 2012). To enable peroxisomal membranes to function in cellular redox regulatory networks, they need to be capable of channelling small redox molecules such as GSH to the cytosol and other cell compartments. In mammalian peroxisomes, there is some evidence to show that GSH can freely diffuse from the cytosol to peroxisomes through a non-selective pore-forming peroxisomal membrane (Nordgren and Fransen, 2014), and GSNO may do so using a similar system. However, in plant peroxisomes the mechanism by which GSH is transported into and out of peroxisomes has not yet been established (Noctor *et al.*, 2011). There is some evidence to show that changes in the redox state or accumulation of H₂O₂ in peroxisomes can alter the redox state in the cytosol (Fig. 2). Hydrogen peroxide produced during photorespiration in catalase-deficient arabidopsis plants (*cat2*) increases total GSH content, mainly in the oxidized (GSSG) form (Mhamdi *et al.*, 2010). As this change in GSH may be involved in transmitting H₂O₂ signals generated in peroxisomes, it may therefore be an important component of the redox signalling network (Mhamdi *et al.*, 2012). The accumulation of GSH in catalase-deficient plants mainly takes place in the vacuoles, chloroplasts and, to a lesser extent, the cytosol (Mhamdi *et al.*, 2012). Transcriptomic studies carried out on *cat2* mutants suggest that cytosolic components of the ASC–GSH system are upregulated (Mhamdi *et al.*, 2010), probably in order to cope with excess H₂O₂ that escapes from the peroxisome to the cytosol. In animal cells, inhibition of catalase activity interferes with redox homeostasis and ROS metabolism in mitochondria and impairs cell growth and ageing (Nordgren and Fransen, 2014). Cytosolic redox changes influence the dual targeting of 6-phosphogluconolactonase 3 (PGL3) to chloroplasts and peroxisomes in arabidopsis leaves, a process requiring thioredoxin m2 (Trxm2) (Hölscher *et al.*, 2014). Whether these redox changes are regulated by peroxisomal signals has not been demonstrated.

PEROXISOMES REGULATE CELL RESPONSES UNDER STRESS CONDITIONS AND DURING DEVELOPMENT

Considerable advances have been made in the last 10 years that underpin our current knowledge and understanding of the role of ROS and NO as intracellular and intercellular messengers with a broad spectrum of regulatory functions in many physiological processes and stress responses. By promoting oxidative and nitrosative stress, the accumulation of ROS and RNS can be deleterious, and their role in signalling networks depends on the finely tuned regulation of their synthesis and degradation.

ROS production in peroxisomes is associated with metabolic pathways such as photorespiration, ureide metabolism and fatty acid β -oxidation, with disturbances in any of these processes giving rise to changes in the accumulation of ROS. This situation could be perceived by the cell as an alarm bell triggering a whole chain of events to boost defence responses. The specific accumulation of ROS in a cell compartment produces an ROS signature capable of triggering a specific response (Mittler *et al.*, 2011; Rosenwasser *et al.*, 2011; Sewelam *et al.*, 2014), although the mechanism and components involved in recognizing and transmitting the information to the nucleus are unclear. The role of H₂O₂ as a signalling molecule is based on the activation of transcription factors and intracellular localization of numerous signalling proteins by redox changes in cysteine or S–Fe clusters (Yun *et al.*, 2011). However, peptides derived from the proteolytic breakdown of oxidatively damaged proteins may also act as secondary ROS messengers regulating specific genes and thus contribute to retrograde ROS signalling (Møller and Sweetlove, 2010), although this hypothesis has not yet been confirmed.

Genetic and pharmacological techniques have demonstrated that peroxisomal H₂O₂ can affect nuclear gene expression (Takahashi *et al.*, 1997; Gechev *et al.*, 2005; Vanderauwera *et al.*, 2005; Queval *et al.*, 2007). It is possible to speculate that peroxisomal NO also regulates gene expression. However, this is difficult to demonstrate as the peroxisomal enzyme involved in NO production has not yet been identified and no specific inhibitors of peroxisomal NO production are available.

Catalase loss-of-function mutants have proved to be an excellent tool for studying the signalling pathway dependent on peroxisomal H₂O₂ using transcriptomic analyses, which have identified hundreds of genes regulated by peroxisomal H₂O₂ associated with photorespiration (Vandenabeele *et al.*, 2004; Vanderauwera *et al.*, 2005; Queval *et al.*, 2007; Inzé *et al.*, 2012). In general, down-regulation of *CAT* makes cells more sensitive to oxidative stress (Mhamdi *et al.*, 2012), with stress symptoms differing according to photoperiod and CO₂ content. Arabidopsis *cat2* lines show salicylic acid (SA)-dependent lesions and induction of pathogenesis-related proteins under long-day but not short-day conditions (Chaouch *et al.*, 2010), differences that have been associated with distinct transcriptomic signatures (Queval *et al.*, 2012). Induction of pathogen-associated processes has also been observed in catalase-deficient tobacco plants (Takahashi *et al.*, 1997). Genetic studies using double mutants that affect catalase activity and some components of the ASC–GSH cycle in the cytosol have facilitated the study of the interplay between catalase and other

antioxidants. Arabidopsis and tobacco double mutants deficient in *CAT* and *APX* are more tolerant to stress than single mutants (Rizhsky *et al.*, 2002; Vanderauwera *et al.*, 2011). In the arabidopsis double mutant deficient in *APX1* and *CAT2*, a specific acclimation response was triggered involving DNA repair activation, cell cycle regulation and anti-programmed cell death mechanisms (Vanderauwera *et al.*, 2011). Transcriptomic analyses of arabidopsis mutants deficient in *GRI* and *CAT2* showed some similarities in the gene expression profile, while the double mutant showed exacerbated oxidative stress (Mhamdi *et al.*, 2010). However, it is important to note that *GRI* encodes a protein targeting cytosol and peroxisomes (Kataya and Reumann, 2010). The cytosolic NADP isocitrate dehydrogenase may modulate responses in *cat2*, while the peroxisomal NADPH isocitrate dehydrogenase does not appear to make any significant contribution (Mhamdi and Noctor, 2015). This further confirms the important role played by cytosolic redox homeostasis in the regulation of peroxisome-dependent H₂O₂ signalling. Recently, a new component of the peroxisomal-dependent H₂O₂ network was identified by using arabidopsis double mutants *cat2* and *pp2a-b'c* (2A protein phosphatase subunit), which demonstrates that PP2A-B'γ is a major player in controlling daylength-dependent responses to oxidative stress (Li *et al.*, 2014). PP2A-B'γ may be involved in regulating ROS production in the plasmalemma while phytochrome A could constitute a nexus between oxidative stress, daylength, protein phosphorylation/dephosphorylation and pathogenesis responses (Li *et al.*, 2014). The existence of a strong relationship between plasmalemma/apoplastic ROS production and peroxisomal H₂O₂-dependent signalling in response to pathogens has also been demonstrated in the double mutant *cat2 AtrbohF* (Chaouch *et al.*, 2012). Calcium ions have emerged as an important component of ROS-dependent signalling in peroxisomes. These organelles contain an intra-organelle store of Ca²⁺, which can contribute to the regulation of H₂O₂ accumulation in peroxisomes by activating catalases (Costa *et al.*, 2010) and may also regulate NO production in peroxisomes.

Comparison of different microarray data sets profiling the arabidopsis transcriptome under conditions that promote high levels of photorespiratory H₂O₂ revealed that the expression of a total of 783 transcripts changed. Most of these modifications were associated with biotic and abiotic stress responses, the largest overlap being observed with respect to heat and osmotic stress (Foyer *et al.*, 2010; Inzé *et al.*, 2012). Analysis of the sub-cellular localization of these H₂O₂-induced proteins points to the nucleus and cytosol as the principal targets, with some being transcription factors or related to nucleic acid binding (Inzé *et al.*, 2012). Transcriptomic techniques have also demonstrated close interaction between peroxisomal H₂O₂, oxidative stress and phytohormone-dependent signalling involving ethylene, auxins, JA, SA and abscisic acid, suggesting that redox homeostasis, particularly the GSH/GSSG ratio, may modulate this relationship (Queval *et al.*, 2007; Tognetti *et al.*, 2012; Mhamdi *et al.*, 2010, 2012). Recently, interaction between photorespiratory H₂O₂ and auxin signalling has been reported (Fig. 2). Thus, the supply of natural or synthetic IAA to arabidopsis *cat2* mutants inhibits photorespiration-dependent cell death induced by growing plants under continuous light (Kerchev *et al.*, 2015). Downregulation of xanthine dehydrogenase (XDH) in *xdh* arabidopsis plants prevents the epinasty

phenotype induced by the auxinic herbicide 2,4-D, which suggests interaction between peroxisomal ROS and auxin signalling (Pazmiño *et al.*, 2014; Rodríguez-Serrano *et al.*, 2014).

The use of arabidopsis mutants deficient in GOX has facilitated the study of the contribution of H₂O₂ from different GOX isoforms to the regulation of cell responses to infection with *Pseudomonas* (Rojas *et al.*, 2012). Hydrogen peroxide, specifically generated by hydroxy acid oxidase 2 (HAOX2) and GOX3, activated components of the SA signal transduction cascade and also appeared to regulate the JA and ethylene pathways involved in cell responses to infection, while GOX1 and GOX2 only played a secondary or indirect role in defence responses (Rojas *et al.*, 2012). Hydrogen peroxide from GOX may constitute a second oxidative wave after 24 h of inoculation of tobacco and arabidopsis plants and could trigger a defence response different from that regulated by NADPH oxidases (Rojas *et al.*, 2012). The increase in GOX and glyoxylate aminotransferase activities is also associated with the hypersensitive response in *Cucumis melo* line P1 infected with the oomycete *Pseudoperonospora cubensis* (Taler *et al.*, 2004).

PEROXISOMES CAN ACT AS SENSORS OF CHANGES IN ROS LEVELS IN THE CELL

Signalling and metabolic networks have usually been studied in terms of changes in metabolites, signal molecules and gene expression without taking into account that organelles can change their dynamic properties and communicate with each other in response to their environment. Organelle movement is necessary for cell functionality, including cross-communication between organelles and for signalling (Rodríguez-Serrano *et al.*, 2009; Suzuki *et al.*, 2012). Peroxisomes are highly dynamic organelles, the number of which can increase under stress conditions induced by ozone, light, xenobiotics, salinity and metals in a process termed proliferation (Oksanen *et al.*, 2003; Mitsuya *et al.*, 2011; Sandalio *et al.*, 2013). Peroxisome proliferation takes place through a complex process involving elongation, constriction and fission (Hu *et al.*, 2012). One of the most challenging topics in peroxisome research is to decipher the signalling pathways governing the regulation of this process under different environmental and metabolic conditions. Some evidence suggests that proliferation of peroxisomes is governed by ROS, given that peroxisomal biogenesis genes are regulated by H₂O₂ in both plant and animal cells (López-Huertas *et al.*, 2000). This has led to the suggestion that peroxisomal proliferation may be a mechanism of protection against oxidative stress. However, arabidopsis and tobacco mutants showing constitutive proliferation of peroxisomes do not show greater resistance to infection or salt treatment, which is probably due to the imbalance in redox homeostasis (Mitsuya *et al.*, 2011; Valenzuela-Soto *et al.*, 2011). Peroxisomal extensions, or peroxules, can be observed under oxidative stress conditions; although their precise function is unclear, it has been suggested that they are involved in the elongation of peroxisomes (Sinclair *et al.*, 2009). The speed at which peroxisomes move can also change under different conditions. In arabidopsis plants exposed to cadmium, an increase in peroxisomal speed has been reported to be regulated by ROS and Ca²⁺ (Rodríguez-Serrano *et al.*, 2009). However, the role played by

these changes in the dynamics of peroxisomes has not been clearly established, with several functions having been suggested: (1) to improve H₂O₂ detoxification in different parts of the cell; (2) to aid signalling processes; and (3) to facilitate the import of new proteins needed for defence purposes (Rodríguez-Serrano *et al.*, 2009). Thus, peroxisomes can act as sensors of ROS/redox changes in the cell and probably trigger a rapid and very specific response to environmental cues, although this needs to be studied in greater depth. The presence of peroxisomal ROS/RNS and redox signal receptors has not been reported in plant tissues. A recent study of mammalian cells has shown that a cytosolic protein containing valosin (VCP), belonging to the AAA class of ATPases, can sense H₂O₂ through redox changes in a cysteine group and interacts with PEX19 (required for peroxisome biogenesis), which regulates the import of catalase into the peroxisome (Murakami *et al.*, 2013). In yeast and human fibroblasts, peroxin 5 (PEX5), a receptor of peroxisomal matrix proteins, has been reported to act as a redox switch regulating the import of these proteins into peroxisomes (Ma *et al.*, 2013; Nordgren and Fransen, 2014). Identification of redox-sensitive proteins involved in peroxisomal protein import and proliferation in plants is a key element in understanding the signalling network that governs the rapid response to environmental changes and the role of ROS/RNS in this process.

In order to maintain cellular homeostasis, the proliferation of peroxisomes needs to be controlled, meaning that excess peroxisomes have to be removed. In mammals and fungi, peroxisomes can be degraded by selective autophagy (pexophagy), which requires sequestration of the organelles in autophagosome compartments and further degradation in the vacuole (Baker and Paudyal, 2014). There is growing evidence to support the idea that autophagy may also play an important role in controlling peroxisomal quality in plants through the selective degradation of oxidized peroxisomes (Shibata *et al.*, 2014), in which peroxisomal protease LON2 plays a key role (Shibata *et al.*, 2013; Baker and Paudyal, 2014). Various genetic studies using the arabidopsis mutant *lon2*, which is defective in AUTOPHAGY-RELATED (ATG) genes (*atg2*, *atg3*, *atg5*, *atg7* and *atg8*), have demonstrated that autophagy is involved in peroxisomal turnover and can be induced by H₂O₂ and oxidative damage to peroxisomes (Shibata *et al.*, 2013; Baker and Paudyal, 2014). Catalases have been reported to act as a possible molecular link between ROS and the promotion of autophagy-dependent cell death (Hackenberg *et al.*, 2013). This mechanism operates independently of the degradation of peroxisome-associated proteins described previously, although, LON2 plays an important role in both cases (Baker and Paudyal, 2014). The specific peroxisomal receptor involved in the interaction with ATG8 to initiate pexophagy has not yet been identified in plants. However, in animals, LC3-II (homologous to the plant *ATG8*) is bound to PEX14, which points to the possibility that *AtPEX14* alleles may be involved in this process (Baker and Paudyal, 2014).

CONCLUSIONS

The presence of different sources of ROS and RNS in peroxisomes, some of which are associated with important metabolic pathways, in addition to the complex battery of antioxidants

present in these organelles, demonstrates the important role of peroxisomes in cellular oxidative metabolism. Although peroxisomes can be a source of stress when over-accumulation of ROS/RNS takes place, they can also act as sensors of oxidative stress induced by different stimuli and by regulating target genes involved in cell responses. In addition to ROS and RNS, peroxisomes also participate in hormone biosynthesis, which in turn may play an important role in the network regulating gene transcription in response to environmental cues (Fig. 2). Peroxisomes can thus be regarded as not merely refuse-collecting organelles, but also as a highly important decision-making platform in the cell. Future research on peroxisomes should aim to elucidate the molecular mechanisms underlying the way in which peroxisomes perceive different signals and regulate their metabolism, morphology and proliferation, as well as the role of ROS/RNS and post-translational modifications of peroxisomal proteins in these processes. Cross-talk between peroxisomes, mitochondria and chloroplasts and the ROS/RNS-dependent signalling events governing these interactions is another exciting field in cell biology that should be studied in depth in order to understand the regulation of development and cell responses to the environment.

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