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REVIEW

SPECIAL ISSUE: PLANT CELL BIOLOGY

Peroxisomal plant metabolism – an update on nitric oxide, Ca^{2+} and the NADPH recycling network

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ABSTRACT

Plant peroxisomes are recognized organelles that – with their capacity to generate greater amounts of H_2O_2 than other subcellular compartments – have a remarkable oxidative metabolism. However, over the last 15 years, new information has shown that plant peroxisomes contain other important molecules and enzymes, including nitric oxide (NO), peroxynitrite, a NADPH-recycling system, Ca^{2+} and lipid-derived signals, such as jasmonic acid (JA) and nitro-fatty acid (NO_2-FA). This highlights the potential for complex interactions within the peroxisomal nitro-oxidative metabolism, which also affects the status of the cell and consequently its physiological processes. In this review, we provide an update on the peroxisomal interactions between all these molecules. Particular emphasis will be placed on the generation of the free-radical NO, which requires the presence of Ca^{2+}, calmodulin and NADPH redox power. Peroxisomes possess several NADPH regeneration mechanisms, such as those mediated by glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) proteins, which are involved in the oxidative phase of the pentose phosphate pathway, as well as that mediated by NADP-isocitrate dehydrogenase (ICDH). The generated NADPH is also an essential cofactor across other peroxisomal pathways, including the antioxidant ascorbate–glutathione cycle and unsaturated fatty acid β-oxidation, the latter being a source of powerful signaling molecules such as JA and NO_2-FA.

KEY WORDS: Ca^{2+}, NADPH, Nitric oxide, NADP-dehydrogenases, Peroxisome

Introduction

Peroxisomes were first described at the cellular level in animal and plant organs in the 1950s and 1960s (Rhodin, 1954; Frederick et al., 1968), when their biochemical composition also began to be analyzed. They have been shown to have a basic oxidative metabolism, characterized by high hydrogen peroxide (H_2O_2) content and the presence of catalase (De Duve et al., 1960; Tolbert et al., 1968). This enzyme, which is exclusively found in peroxisomes, is considered to be one of the main antioxidant enzymes and part of the first line of defense against oxidative stress. Subsequent advances in the study of these organelles have revealed that they are extraordinary subcellular compartments characterized by great endogenous metabolic adaptability and highly active interactions with other subcellular compartments (van der Zand et al., 2006; Mast et al., 2015; Wanders et al., 2016). This adaptability is even more remarkable for plant peroxisomes, because their enzymatic composition reflects the functionality of the respective tissue and can change depending on the cell type in each plant organ (root, cotyledon or leaf), the stage of development or external conditions (Goto-Yamada et al., 2015; Reumann and Bartel, 2016). An excellent example of plant peroxisome adaptability and flexibility is the dynamic and reversible interactions between leaf peroxisomes and chloroplasts, which varies depending on illumination conditions. Here, the external appearance of peroxisomes changes from a spherical (dark conditions) to an elongated elliptical (light conditions) shape in order to increase the contact surface with chloroplast, thus facilitating effective metabolite interchange (Oikawa et al., 2015; Corpas, 2015). The use of new biochemical, proteomic and cellular approaches has increased our knowledge of the components and functions of plant peroxisomes (Palma et al., 2009; Hu et al., 2012; Kataya et al., 2015; Reumann and Bartel, 2016; Cui et al., 2016; Corpas et al., 2017). Furthermore, H_2O_2 and nitric oxide (NO) are not only found in chloroplasts and mitochondria, but are also present in peroxisomes. This raises the question as to whether they function as signals between the different cellular compartments. This review provides an update on the metabolic interconnections between some of the newly identified components in peroxisomes, including NO, Ca^{2+}, NADPH and nitro fatty acid (NO_2-FA). Thus, the involvement of enzymes, such as NADP-dehydrogenases (NADP-DHs), NAD kinases (NADKs) and Nudix hydrolase 19 (NUDX19, also known as NUDT19), contributes to the regulation of peroxisomal redox status mediating a change in the NADPH:NADP and GSH:GSSG ratio. These molecules are also connected to the enzymatic generation of NO, which involves NADPH, calmodulin (CaM) and Ca^{2+}. In addition, the presence of lipid-derived signals, such as jasmonic acid (JA) and NO_2-FA, opens up new avenues of research into the importance of peroxisomes and their interaction with other cellular compartments.

Peroxisomal NO generation depends on Ca^{2+} and CaM

NO generation in peroxisomes

NO is a versatile molecule involved in numerous physiological processes and in the mechanism of defense against both biotic and abiotic stresses (Domingos et al., 2015; Simontacchi et al., 2015). Given the prominence of peroxisomal oxidative metabolism over the last 15 years, research has especially focused on the potential presence of NO and related molecules called reactive nitrogen species (RNS) in peroxisomes. We have analyzed the generation of this molecule and its modulation of proteins whose function in peroxisomes is modified upon post-translational modification, such as nitration and *S*-nitrosation (Corpas and Barroso, 2014a; Chaki et al., 2015; Begara-Morales et al., 2016; Corpas et al., 2017), as well as its potential role as a signaling molecule promoting

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intracellular communication. NO has been detected in peroxisomes in different plant species using techniques such as spin trapping electron paramagnetic resonance (EPR) spectroscopy and fluorescence probes (Corpas et al., 2004, 2009). In addition, there is biochemical evidence to show that peroxisomal NO is generated by an L-arginine-dependent NO synthase (NOS)-like activity in plant cells (Barroso et al., 1999; Corpas and Barroso, 2016). Interestingly, NOS proteins have also been detected in animal peroxisomes (Stolz et al., 2002), suggesting that this enzyme is a constitutive component of peroxisomes, although the identity of the plant NOS protein is still unknown. However, it is known that the NOS protein contains a type 2 peroxisomal-targeting signal (PTS2), which is imported into the peroxisomal matrix; this domain is present in enzymes responsible for NO generation in plant and animal systems (Loughran et al., 2013; Corpas and Barroso, 2014b). Plant peroxisomal NOS-like activity has been found to require additional molecules – especially NADPH, Ca^{2+} and CaM, similar to what is found during the regulation of NOS activity in animal cells (Barroso et al., 1999; Corpas et al., 2004; Alderton et al., 2001). Peroxins (PEXs), which are peroxisomal biogenesis factors located in the cytosol and the peroxisomal membrane itself, are involved in matrix protein transport (Cross et al., 2016). In addition, the import of peroxisomal NOS proteins requires PEX12, as well as both of the PTS2 receptors PEX5 and PEX7. Through the use of Ca^{2+} channel blockers and CaM antagonists, we have also shown that this import is strictly dependent on both Ca^{2+} and CaM (Corpas and Barroso, 2014b). Fig. 1 shows a working model of the known elements involved in the peroxisomal protein import mechanism responsible for NO generation.

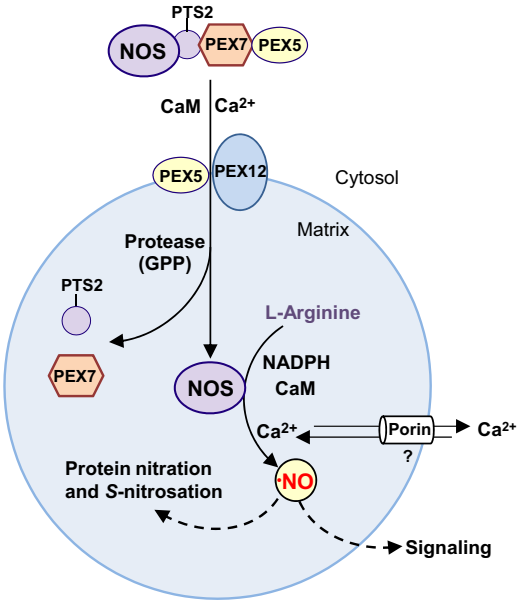

Fig. 1. Schematic model of known elements involved in the import mechanism of NOS which generates NO in the peroxisome. The NOS protein contains a PTS2 domain. Import of the peroxisomal NOS protein requires Ca^{2+}, CaM and the involvement of several peroxins (PEXs), including PEX12, which is located in the membrane, and both the PTS receptors (PEX5 and PEX7), located in the cytosol. Following the import of the NOS protein into the matrix, PTS2 is cleaved by a glyoxysomal-processing protease (GPP). Ca^{2+} needed for NOS activity could move through a porin ion channel located in the peroxisomal membrane. NO can mediate post-translational modifications (nitration and *S*-nitrosation) and functions as a signaling molecule outside the peroxisomes. Dashed lines with arrow indicate the signaling processes. This figure has been modified Corpas and Barroso (2014b) with permission from Wiley © 2015 Federation of European Biochemical Societies.

Ca^{2+} and CaM function in plant peroxisomes

It is well established that Ca^{2+} and CaM are key regulatory elements in many cellular processes in animals and plants (Hepler, 2005; Clapham, 2007; Perochon et al., 2011). The presence of these factors in plant peroxisomes, as well as their potential metabolic functions, has recently begun to be studied. Ca^{2+} can move through membranes using voltage-dependent anion channels, among others (Gincel et al., 2001; Jammes et al., 2011). These channels are a class of porin ion channel, and their presence has been described in peroxisomal membranes (Fig. 1) (Reumann et al., 1997, 1998; Corpas et al., 2000). However, the presence of Ca^{2+} and its effect on catalase have only recently been assessed in plant peroxisomes (Costa et al., 2010; Yang and Poovaiah, 2012). The presence of Ca^{2+} also closely correlates with that of Ca^{2+}-dependent protein kinase (CDPK) CPK1 in *Arabidopsis* peroxisomes. CPK1 appears to be not only involved in lipid metabolism and oxidative stress (Dammann et al., 2003), but also in mediating pathogen resistance (Coca and San Segundo, 2010). Similarly, CDPK2, which is involved in pollen tube growth, has been found in *Petunia inflata* peroxisomes (Yoon et al., 2006; Guo et al., 2013), which is indicative of a direct involvement of Ca^{2+} and peroxisomal NO in this process (Prado et al., 2004, 2008).

Additionally, *Arabidopsis* peroxisomes have been reported to contain the calmodulin-like (CML) protein CML3 (Chigri et al., 2012). CML3 can modulate Ca^{2+} signals, as it contains amino acid sequences commonly found in canonical CaM-binding motifs (Perochon et al., 2011). It mediates the dimerization of the *Arabidopsis thaliana* peroxisomal ATP-independent serine endopeptidase (DEG15), which belongs to the family of degradation of periplasmic protein (DEG) proteases; these are also orthologs of glyoxysomal-processing protease (GPP) in the watermelon (Helm et al., 2007). In its monomeric form, GPP exerts general protease activity; in its dimeric form, it is capable of specifically cleaving PTS2 domains (Fig. 1) (Schuhmann et al., 2008; Dolze et al., 2013). The PTS2 domain is present in plant peroxisomal enzymes, such as malate dehydrogenase, citrate synthase, acyl-CoA oxidase and thiolase; after cleavage by this protease, the proteins become functional (Mullen, 2002; Johnson and Olsen, 2003; Pracharoenwattana et al., 2007; Meng et al., 2014).

Recently, we reported that different CaM antagonists cause a reduction in peroxisomal Ca^{2+} levels, which adversely affects peroxisomal protein import (Corpas and Barroso, 2017b). Several peroxisomal enzymes, including catalase, hydroxypyruvate reductase (HPR) and glycolate oxidase (GOX), involved in photorespiration, were also found to be downregulated upon such treatment (Corpas and Barroso, 2017b). GOX is an important source of H_2O_2, suggesting that there is a close correlation between Ca^{2+} levels and H_2O_2. Therefore, it is plausible to assume that peroxisomal Ca^{2+} is crucial for normal function of several organelles. Table 1 summarizes the effects of Ca^{2+} on the peroxisomal protein function.

Thus, the newly identified peroxisomal molecules NO, Ca^{2+} and CaM show metabolic interactions, which evokes new questions regarding the physiological role of peroxisomes in both optimal and adverse environmental conditions.

Peroxisomal NADPH recycling and functionality

The reducing power of the NADPH cofactor is well known to be essential for normal cell growth and proliferation. As mentioned previously, NADPH is also necessary as an electron donor for the generation of NO in peroxisomes. In plants, the main source of NADPH in photosynthetic cells is the ferrodoxin-NADP reductase (FNR) enzyme. However, in non-photosynthetic cells, such as roots, or in photosynthetic cells during the dark phase, a family of

Table 1. Plant peroxisomal proteins that modulate and are affected by Ca^{2+} content

Enzyme	Cellular process	Reference
Ca^{2+}-binding proteins		
Ca-dependent protein kinase (CPK)		
CPK1 (*Arabidopsis*)	Pathogen resistance	Coca and San Segundo, 2010
CDPK2 (*Petunia inflata*)	Pollen tube growth	Yoon et al., 2006; Guo et al., 2013
Calmodulin-like protein 3 (CML3)	Mediates dimerization of peroxisomal processing protease DEG15 to cleave PTS2	Helm et al., 2007
Proteins affected by Ca^{2+}		
NO synthase-like activity	Generation of NO	Barroso et al., 1999; Corpas et al., 2004
Catalase	H_2O_2 degradation	Costa et al., 2010; Yang and Poovaiah, 2012
Hydroxypyruvate reductase (HPR)	Photorespiration	Corpas and Barroso, 2017a,b
Glycolate oxidase (GOX)	Photorespiration	Corpas and Barroso, 2017a,b

NADP-DHs regenerates NADPH. These NADP-DHs include two enzymes of the oxidative step of the pentose phosphate pathway, the glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) proteins, as well as the NADP-malic enzyme (NADP-ME), also known as NADP-malate dehydrogenase, and NADP-isocitrate dehydrogenase (NADP-ICDH) (Corpas and Barroso, 2014c). All these NADP-DHs have different isozymes that are present in the respective subcellular compartments, including the cytosol, the chloroplast, mitochondria and peroxisomes.

NADP-DHs in peroxisomes

A set of NADPH-recycling enzymes have been detected in plant peroxisomes, including the above mentioned NADP-ICDH (Corpas et al., 1999; Reumann et al., 2007; Leterrier et al., 2016), as well as G6PDH and 6PGDH proteins (Corpas et al., 1998; Fernández-Fernández and Corpas, 2016; Hölscher et al., 2016) (see also Table 2). These NADP-DHs operate in coordination with certain import mechanisms in order to supply the substrate corresponding to each enzyme (Fig. 2). With regard to isocitrate, which is necessary for NADP-ICDH activity, the most plausible import mechanism is through the isocitrate–2-oxoglutarate shuttle (Reumann, 2000; Rottensteiner and Theodoulou, 2006; Linka and Theodoulou, 2013). However, the import system for glucose 6-phosphate (G6P), which is used by the G6PDH enzyme, has – to our knowledge – not been described in the peroxisomal membrane. One suspects the presence of a G6P translocator, similar to what is known to occur in plastids (Kunz et al., 2010). Nevertheless, an alternative import route for G6P could be through a porin in the peroxisomal membrane (Fig. 2) (Reumann et al., 1998).

Another way to maintain NADPH regeneration is to provide an appropriate $NADP^+$ pool, which can be affected through the NAD^+ metabolism. Recently, the membrane-located peroxisomal NAD^+ carrier (PXN), which enables NAD^+ to be imported in exchange for adenosine monophosphate (AMP), has been described in *Arabidopsis* (Bernhardt et al., 2012; van Roermund et al., 2016). In addition, NAD kinase (NADK) activity is known to catalyze the direct phosphorylation of NAD^+ to $NADP^+$ (Pollak et al., 2007) (Fig. 2). Of the three NADKs identified in *Arabidopsis* (Turner et al., 2005), NADK3 has been reported to be localized in the peroxisomal matrix (Waller et al., 2010), where it contributes to the generation of the peroxisomal $NADP^+$ pool (Chai et al., 2006; Li et al., 2014) and maintains peroxisomal NADP-DH activity (Fig. 2).

In addition to the capacity to regenerate NADPH, the peroxisomal NADP-DHs are involved in other potential biological processes, such as leaf senescence, stomata movements and pollen-tube–ovule interactions. For example, leaf senescence is a highly regulated process characterized by antioxidative capacity loss, in which peroxisomes are involved (Pastori and del Río, 1997; Jiménez et al., 1998; Zimmermann et al., 2006). With regard to peroxisomal NADP-ICDH activity, a comparative analysis of its kinetic parameters in young and senescent leaves of pea plants has revealed that the NADP-ICDH affinity (K_m) for their isocitrate substrate decreased drastically during leaf senescence. This leads to over a 20-fold increase in isocitrate affinity, while the maximum rate (V_{max}) remains unchanged (Corpas et al., 1999). There are two possible explanations for this finding: first, that this allows the peroxisomal intracellular pool of isocitrate to outcompete the glyoxylate cycle enzyme isocitrate lyase, which appears in peroxisomes during leaf senescence (Pastori and del Río, 1997);

Table 2. Plant peroxisomal enzymes that consume or regenerate NADPH

Enzyme	Biochemical pathway and function	Reference
NADPH-consuming		
2,4-dienoyl-CoA reductase (DECR)	β-oxidation pathway; specifically participates in the degradation of unsaturated fatty acids	Behrends et al., 1988; Goepfert and Poirier, 2007
NO synthase-like activity	Generation of nitric oxide; signaling functions and mediates post-translational modifications (*S*-nitrosation and nitration)	Barroso et al., 1999; Begara-Morales et al., 2016
Glutathione reductase (GR)	Ascorbate–glutathione pathway; GR allows the regeneration of the antioxidant GSH	Jiménez et al., 1998; Romero-Puertas et al., 2006
12-oxophytodienoate reductase (OPR3)	Biosynthesis of JA; involved in plant defense	Schaller et al., 2000
Nudix hydrolase 19 (NUDX19)	Regulation of NADPH content	Yoshimura and Shigeoka, 2015; Maruta et al., 2016
NADPH-recycling		
Isocitrate dehydrogenase (NADP-ICDH)	Nitrogen metabolism and redox control; stomatal movement	Corpas et al., 1999; Leterrier et al., 2016
Glucose-6-phosphate dehydrogenase (G6PDH)	Pentose phosphate pathway; support of antioxidant system and NO generation	Corpas et al., 1998
6-Phosphogluconate dehydrogenase (6PGDH)	Pentose phosphate pathway; pollen-tube–ovule interaction	Hölscher et al., 2016

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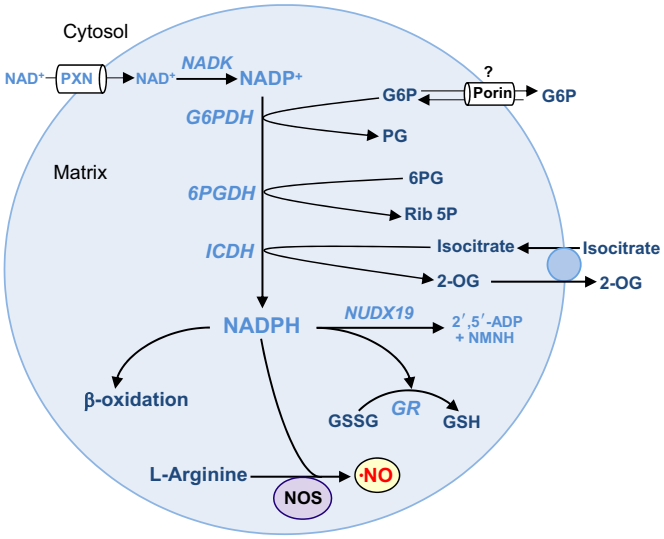

Fig. 2. Schematic representation of peroxisomal NADPH recycling pathways. NADPH is regenerated by several NADP-DHs, including G6PDH, 6PGDH and NADP-ICDH. The substrates for these dehydrogenases may be imported through different mechanisms: through a porin for G6P or through an isocitrate–2-oxoglutarate (2-OG) shuttle in the case of isocitrate. Both are located in the peroxisomal membrane. NADPH is used in several processes, including β-oxidation, NO generation by an L-arginine-dependent NOS activity and conversion of GSSG into GSH by GR activity. NADPH could be hydrolyzed to reduced nicotinamide mononucleotide (NMNH) plus 2′,5′-ADP by NUDX19 activity. The $NADP^+$ pool is provided by NAD^+ phosphorylation through NADK activity. NAD^+ may be imported into the peroxisomal matrix by a NAD^+ carrier (PXN) present in the membrane. PG, 6-phosphogluconolactone; 6PG, 6-phosphogluconate; Rib 5P, ribulose 5-phosphate.

or second, that the increase provides a larger supply of NADPH to eliminate excess H_2O_2 produced during senescence, when catalase activity decreases sharply (Corpas et al., 1999; Zimmermann et al., 2006). Stomatal movement is regulated by a complex network of signals, including Ca^{2+}, NO, H_2O_2 and phytohormones (abscisic acid, JA and salicylic acid) (Murata et al., 2015). Recently *Arabidopsis* null mutants for the peroxisomal NADP-ICDH were shown to have stomatal movement malfunction (Leterrier et al., 2016). In this regard, it is worth pointing out that senescence and stomatal movement are also regulated by NO levels in peroxisomes (Corpas et al., 2004; Leterrier et al., 2016). Another example is peroxisomal 6PGDH in *Arabidopsis*, whose activity is necessary during growth and developmental processes, such as male gametophyte growth and pollen-tube–ovule interaction (Hölscher et al., 2016). Interestingly, Ca^{2+} and peroxisomal NO are also directly involved in the latter processes (Prado et al., 2004, 2008).

Function of peroxisomal NADPH

Apart from the involvement of NADPH in generating NO, as mentioned above, this reducing agent is also required for several other peroxisomal enzymatic activities that are involved in various pathways (see Table 2). For example, 2,4-dienoyl-CoA reductase (DECR) activity catalyzes the conversion of 2-trans,4-trans-dienoyl-CoA+NADPH into 3-trans-enoyl-CoA+$NADP^+$ (Behrends et al., 1988; Goepfert and Poirier, 2007). This peroxisomal DECR is part of the alternative β-oxidation pathway present in plant peroxisomes, which is necessary to degrade unsaturated fatty enoyl-CoA esters with double bonds in even- and odd-numbered carbons (Fig. 3) (Rottensteiner and Theodoulou, 2006). Furthermore, 12-oxophytodienoate reductase (OPR) enzymes catalyze the conversion of 12-oxo-phytodienoic acid (OPDA)+NADPH into 3-oxo-2[2′(Z)-pentenyl]-cyclopentane-1-octanoate (OPC-8:0) and $NADP^+$. In *Arabidopsis*, OPR3 is located in peroxisomes and is involved in JA biosynthesis, which has a role in signaling during development and in the response to adverse stresses, such as mechanical wounding and pathogens (Schaller et al., 2000; Strassner et al., 2002; Baker et al., 2006; Tani et al., 2008; Gfeller et al., 2010, see also below).

Another peroxisomal enzyme that requires NADPH is glutathione reductase (GR; At3g24170). This enzyme is part of the ascorbate–glutathione cycle, which facilitates the regeneration of the antioxidant glutathione (GSH) in the following reaction: GSSG+NADPH→GSH+$NADP^+$. GR, whose activity has been observed to increase during natural leaf senescence and in response to abiotic stresses, has been detected in different plant species (Jiménez et al., 1998; Romero-Puertas et al., 2006; Kataya and Reumann, 2010). Similarly, its product, peroxisomal GSH, is increased under stress conditions and can reach a concentration of ∼5 mM in *Arabidopsis* leaf peroxisomes (Koffler et al., 2013; Zechmann, 2014). GSH can also react with NO to form *S*-nitrosoglutathione (GSNO), which may function as an intracellular NO reservoir; it mediates post-translational modifications, such as *S*-nitrosation. This affects the function of the protein, as previously described for catalase, ascorbate peroxidase and monodehydroascorbate peroxidase (Begara-Morales et al., 2016). This highlights connections between the peroxisomal metabolism of NADPH, antioxidant molecules and some the NO-related RNS molecules (Barroso et al., 2013; Corpas et al., 2017).

The last group of enzymes involved in the use of NADPH are pyrophosphatases that contain a highly conserved amino acid sequence or Nudix box, $Gx_5Ex_7REUxEExGU$, in which U is a hydrophobic amino acid such as Val, Ile or Leu (Ogawa et al., 2008). NUDX19 hydrolyzes NADPH to reduced nicotinamide mononucleotide (NMNH) plus 2′,5′-ADP. Amino acid sequence analysis suggests that *Arabidopsis* NUDX19 is located in both peroxisomes and chloroplasts (Ogawa et al., 2008; Lingner et al., 2011). Specifically, it has a C-terminal PTS1 tripeptide (SSL), which is regarded as a weak, but effective, type 1 PTS (PTS1). Indeed, the peroxisomal presence of *Arabidopsis* NUDX19 has been experimentally confirmed in *Arabidopsis* (Lingner et al., 2011). Owing to its NADPH pyrophosphohydrolase activity, NUDX19 can modulate the cellular levels and redox states of this pyridine nucleotide, which could be involved in NADP-DH regulation and hormonal signaling. Recent studies demonstrate that the loss of NUDX19 function affects NADPH content and mediates the increased resistance to arsenic and photooxidative stresses in *Arabidopsis* (Corpas et al., 2016; Maruta et al., 2016). The presence of NUDX19 in peroxisomes and chloroplasts points to metabolic and physical interactions during photorespiration between these organelles (Oikawa et al., 2015). Interestingly, in the *Arabidopsis* NUDX19 knockout mutant, a significant increase in all NADP-dehydrogenase activities (NADP-ICDH, G6PDH and 6PGDH) is triggered in roots and leaves, especially under induced arsenic stress (Corpas et al., 2016). This highlights the importance of NUDX19 in NADPH homeostasis in plants.

The cofactor NADPH could therefore be a necessary link in the redox metabolism of peroxisomes and must be regenerated to function normally; NADPH also represents a connection between several pathways, such as NO generation, the antioxidant system and β-oxidation.

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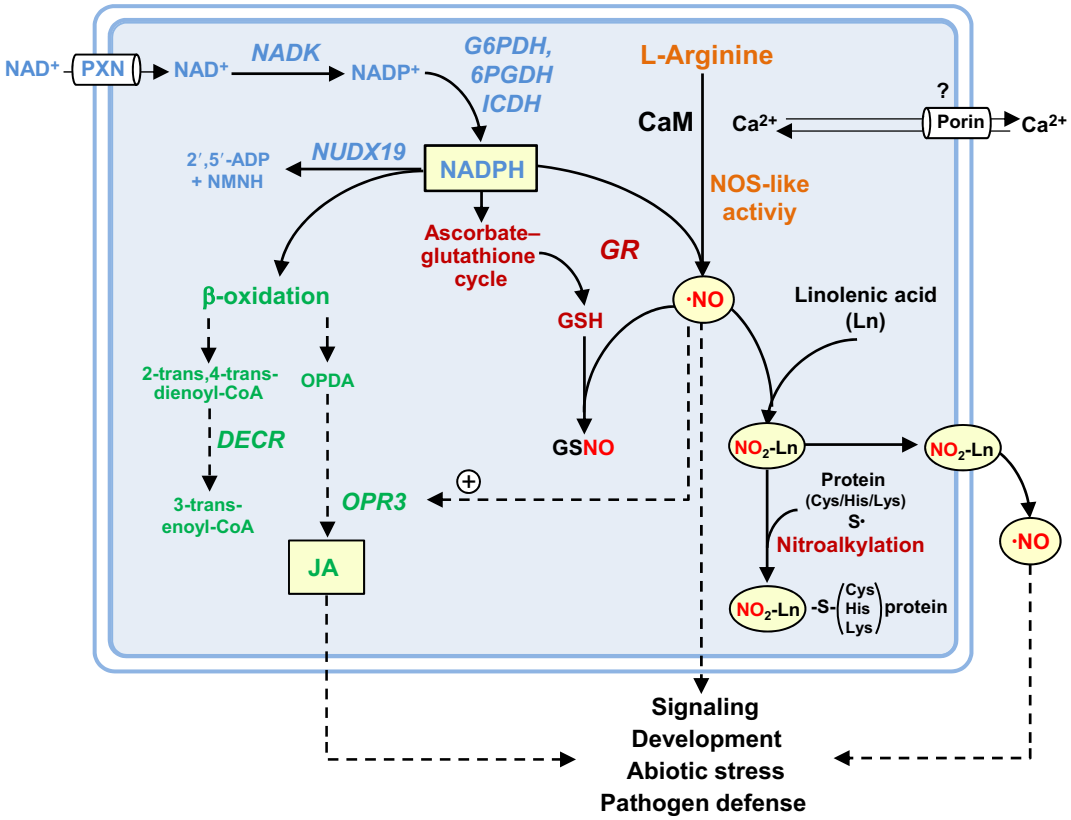

Fig. 3. Model of the metabolic integration of NADPH, NO and Ca^{2+} in plant peroxisomes. The NADPH pool is necessary for different processes, including L-arginine-dependent NOS activity, GSH regeneration through GR activity (part of the ascorbate–glutathione cycle) and β-oxidation. NOS activity requires the presence of Ca^{2+}, CaM and NADPH to generate NO, which can react with reduced glutathione (GSH) to form GSNO, or mediate the formation of NO_2-FAs, such as NO_2-Ln. Alternatively, the electrophilic property of NO_2-Ln may lead to nitroalkylation of proteins containing nucleophilic residues (Cys, His or Lys). This could contribute to the signaling actions, involving NO_2-FAs, and the observed antioxidant properties of these molecules. NO can also function as a signal molecule outside the peroxisomes or positively modulate the expression of OPR3, which mediates JA biosynthesis. NADPH is further needed for DECR activity, which is involved in unsaturated fatty acid β-oxidation. Potential excess NADPH could be hydrolyzed to reduced nicotinamide NMNH plus 2′,5′-ADP by NUDX19 activity. By contrast, the $NADP^+$ pool can be maintained via NAD^+ phosphorylation through NADK activity. NAD^+ may be imported into the peroxisome by a NAD^+ carrier (PXN) present in the membrane. On the other hand, Ca^{2+} could move through a porin ion channel located in the peroxisomal membrane. Peroxisomes contain several NADPH-recycling enzymes, including G6PDH, 6PGDH and NADP-ICDH. OPDA, 12-oxo-phytodienoic acid. Dashed lines with arrow indicate the signaling processes.

Lipid-derived signals – JA and NO_2-FA

Plant peroxisomes house all the components necessary for the β-oxidation of fatty acids (FAs); this oxidation is essential for the mobilization of the lipid reservoir required for seedling development (Graham, 2008). Additionally, peroxisomes are also important for the metabolism of several lipid-derived signals that have a crucial physiological role in the entire plant. The import of FAs into peroxisomes is mediated by ATP-binding cassette (ABC) transporter 1 [ABCC1, also known as COMATOSE (CTS)], which is involved in the import of other molecules such as 12-oxo-phytodienoic acid (OPDA), a precursor of jasmonic acid (JA), as well as 4-dichlorophenoxybutyric acid and indole butyric acid (IBA); the latter of which are precursors of the phytohormone auxin, which is involved in plant growth and development (Baker et al., 2015).

As mentioned above, JA is a linolenic acid-derived cyclopentanone mainly involved in mediating wounding responses (Theodoulou et al., 2005; Delker et al., 2007; Gfeller et al., 2010; León, 2013). JA and its derivatives also modulate a wide range of activities, such as plant development, as well as abiotic (including salinity, drought and UV radiation) and biotic stress tolerance; these activities also provide a defense against pathogen infection and insect attacks (Wasternack and Song, 2017). In this context, the interplay between NO, phytohormones (indoleacetic acid, abscisic acid and ethylene) and other plant growth regulators (polyamines) has been well established (Freschi, 2013; Asgher et al., 2017). This interplay could be of physiological significance in the peroxisomal metabolism, during which these molecules are generated. Indeed, in *Arabidopsis*, there is evidence that NO increases JA production by increasing the expression of the *OPR3* gene in peroxisomes (Fig. 3) (Huang et al., 2004; Mur et al., 2013; Hussain et al., 2016). A similar effect of NO on JA has been observed in tomato roots in response to nematode infection (Zhou et al., 2015). OPR3 activity also affects the metabolism of other molecules, including those of glucosinolates and tryptophan (Cao et al., 2016). Tryptophan is a precursor of serotonin and indoleacetic acid (IAA), which are involved in plant growth and stress responses (Zolman et al., 2008; Schlicht et al., 2013; Spiess and Zolman, 2013). Therefore, apart from the important role played by peroxisomal β-oxidation in lipid mobilization during germination, there are other derived lipids, such as JA, whose synthesis specifically requires NADPH for OPR3 activity. However, expression of the *OPR3* gene is also stimulated by NO, which establishes another connection point between NADPH, JA and NO in peroxisomes (Fig. 3).

NO_2-FAs are derived from NO and produced by the addition of a nitro group (–NO_2) to unsaturated fatty acids. These molecules were first shown to have important physiological functions in animals, such as cardiac ischemic injury protection, regulation of inflammatory processes and reduction of atherosclerosis (Freeman et al., 2008; Trostchansky et al., 2013). In higher plants, the presence of NO_2-FAs was hypothesized a few years ago (Sánchez-Calvo et al., 2013). Recently, we have reported on the endogenous presence and function of NO_2-FAs in *Arabidopsis*, with the most prominent NO_2-FA being nitro-linolenic acid (NO_2-Ln) (Mata-Pérez et al., 2016a). The highest NO_2-Ln content, which diminished during plant development, was found in *Arabidopsis* seeds. NO_2-Ln activates a molecular chaperone network in response to diverse abiotic stresses and has been shown to have a powerful signaling function. NO_2-Ln has the capacity to either increase or decrease the gene expression of hundreds of genes with a remarkable induction capacity, and ∼25% of the total upregulated genes encode heat-shock proteins (Mata-Pérez et al., 2016a). Recently, mass spectrometry analysis of the peroxisomal fraction isolated from the leaves of pea plants has revealed that peroxisomes also contain NO_2-Ln (Mata-Perez et al., 2017). This demonstrates that there is a highly active NO metabolism in plant peroxisomes, as these molecules may function as a NO reservoir with the capacity to release NO (Mata-Pérez et al., 2016b). Alternatively, the electrophilic property of NO_2-Ln could allow it to contribute to nitroalkylation of a protein that contains nucleophilic residues (Cys, His or Lys), which could contribute to the signaling action of NO_2-FAs. Taken together, the physiological function of these molecules in plant peroxisomes raises issues that need to be addressed and could provide new evidence to show that these organelles have a potential signaling function mediated by GSNO and NO_2-FAs.

Conclusions and future perspectives

The metabolism of plant peroxisomes is characterized by adaptability and flexibility, and depends on the organ, stage of development and response to adverse environmental conditions. Consequently, peroxisomes should be regarded as nodes at the crossroads of different metabolic pathways. Examples of these pathways are photorespiration, lipid mobilization and the nitro-oxidative metabolism, which endow cells with signaling molecules, including H_2O_2, NO, GSNO, NO_2-FA and JA. Fig. 3 summarizes a model for the interplay between the various molecules described in this review of plant peroxisomes. Their NADPH content is affected by NADP-DHs, NADKs and NUDX19, which help to regulate the peroxisomal redox status. Moreover, NADPH, synthesis of NO, ascorbate–glutathion cycle and β-oxidation are important for several essential metabolic pathways; NADPH also mediates the generation of lipid-derived signals (JA and NO_2-FA).

However, several points regarding these new peroxisomal molecules need to be addressed in future research: the mechanisms that control their transport through the peroxisomal membrane of Ca^{2+} and the substrates specific to NADP-DHs; the identification of the protein responsible for NO synthesis; the peroxisomal proteins susceptible to post-translational modifications mediated by NO (nitration, *S*-nitrosation or nitroalkylation); the specific function of peroxisomal NO_2-FAs under physiological and stress conditions; and the cellular function of peroxisomal molecules as compared to molecules from other subcellular compartments. The answers to all these points could provide a better understanding of the cellular role of peroxisomes as a source of intra- and inter-cellular signaling molecules (H_2O_2, NO, NO_2-FA and JA). Such an understanding may also be useful for researchers working on peroxisomes in other organisms, including yeast and mammals, in order to provide a more complete picture of the signals involved in biochemical communication between organelles.

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Competing interests

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