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REVIEW PAPER

Nitric oxide is an ubiquitous signal for maintaining redox balance in plant cells: regulation of ascorbate peroxidase as a case study

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Abstract

Oxidative and nitrosative stresses and their respective antioxidant responses are common metabolic adjustments operating in all biological systems. These stresses result from an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) and an imbalance in the antioxidant response. Plants respond to ROS and RNS accumulation by increasing the level of the antioxidant molecules glutathione and ascorbate and by activating specific antioxidant enzymes. Nitric oxide (NO) is a free radical considered to be toxic or protective depending on its concentration, combination with ROS compounds, and subcellular localization. In this review we focus on the mechanisms of NO action in combination with ROS on the regulation of the antioxidant system in plants. In particular, we describe the redox post-translational modifications of cytosolic ascorbate peroxidase and its influence on enzyme activity. The regulation of ascorbate peroxidase activity by NO as a redox sensor of acute oxidative stress or as part of a hormone-induced signalling pathway leading to lateral root development is presented and discussed.

Key words: Ascorbate peroxidase, auxin, nitric oxide, reactive nitrogen species, reactive oxygen species, root development, S-nitrosylation, stress.

Plant stress biology—a redox problem

Plants produce a considerable amount of reactive oxygen species (ROS) as by-products of photosynthetic and respiratory electron transport. Thus, chloroplasts and mitochondria are the main producers of ROS. Under certain conditions that disturb the equilibrium of the electron transport chain or when pigments are overexcited, a single electron is donated to O_2 to form the free radical anion superoxide (O_2^-). Plasma membrane-NADPH oxidase also generates O_2^- (Sagi and Fluhr, 2006). This oxidant agent is highly reactive and a substrate for the generation of other ROS. Superoxide dismutase dismutates O_2^- into hydrogen peroxide (H_2O_2). In turn, H_2O_2 generates hydroxyl radicals ($OH^\cdot$) in combination with metals in a process called the Fenton reaction. These extremely reactive molecules (O_2^-, H_2O_2, $OH^\cdot$) can damage all types of biomolecules. Nevertheless, the idea that ROS are produced uncontrollably in the cell and are simply deleterious is no longer considered to be the case. ROS participate in numerous developmental and regulatory physiological processes in plants, triggering cell responses by acting as signal molecules. To maintain balance, a battery of antioxidant molecules and enzymes operates to reduce the over-accumulation of ROS in the plant cell and apoplast. Together, ROS and these antioxidant components contribute to the redox homeostasis in the cell, playing a fundamental function in the physiological processes in whole plants.

Abbreviations: APX, ascorbate peroxidase; ASC, ascorbate; DHA, dehydroascorbate; GSH, glutathione; GSSG, oxidized glutathione; LR, lateral root; MDA, monodehydroascorbate; NTR, NADPH thioredoxin reductase; NTS, NADPH thioredoxin reductase–thioredoxin system; PCD, programmed cell death; PTM, post-translational modification; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNO, S-nitrosothiol; Trx, thioredoxin; UV, ultraviolet.

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The cellular redox state is defined as the proportion of an antioxidant molecule in its reduced state relative to the total pool size (Potters *et al.*, 2010). The antioxidants ascorbate (ASC) and glutathione (GSH) play major roles in detoxifying oxidative stresses in plants (Foyer and Noctor, 2011). Thus, the redox state in cells can be measured by the ratio of GSH to oxidized GSH (GSSG), and of ASC to dehydroascorbate (DHA, the oxidized form of ASC). Both GSH and ASC, together with NADPH, are substrates of enzymatic activities that control H_2O_2 levels in a series of reactions called the ascorbate–glutathione cycle. For instance, the enzyme ascorbate peroxidase (APX) reduces H_2O_2 to H_2O with ASC as an electron donor [$2ASC + H_2O_2 \rightarrow 2$ monodehydroascorbate (MDA) + $2H_2O$]. Ascorbate is regenerated from MDA by the activity of MDA reductase ($NADH + 2MDA \rightleftharpoons NAD^+ + 2ASC$). The MDA molecules generate ASC and DHA in a disproportionation reaction. DHA is reduced to ASC by dehydroascorbate reductase at the expense of two molecules of GSH ($DHA + 2GSH \rightarrow ASC + GSSG$). Oxidized GSSG is again reduced by GSH reductase at the expense of NADH. GSH has been proposed as a biomarker of the redox state and has been implicated in the direct scavenging of ROS, ASC reduction, detoxification of heavy metals, and meristem redox homeostasis during root growth (Vernoux *et al.*, 2000; Xiang and Oliver, 1998).

Nitric oxide in biological systems: nitric oxide-mediated post-translational modifications of proteins as cellular sensors of oxidative stress

Since the identification of nitric oxide (NO) as an endothelial-derived relaxing factor in mammals (Ignarro, 1989; Palmer *et al.*, 1987), many studies were directed to understanding the redox chemistry of this bioactive molecule. NO is a diatomic gas that can diffuse freely in the cytoplasm and cross lipid bilayers to the extracellular space and *vice versa* (Subczynski *et al.*, 1996). Chemically, NO can accept or donate one electron, alternating between three redox states: the nitroxyl anion (NO^-), the uncharged radical ($NO^\bullet$) with an unpaired electron in the 2p π orbital, and the nitrosonium cation (NO^+). These reactive nitrogen species (RNS) have different chemical properties and reactivity, which explains the diverse reactions occurring in the biological systems in which they participate (Stamler *et al.*, 1992). The main enzymatic sources of NO in plants are: (i) the reductive pathway where nitrite (NO_2) is reduced to form NO, and (ii) the oxidative pathway where substrates such as arginine, polyamines and hydroxylamines are oxidized, leading to NO synthesis. In the reductive pathway, the electron donated to reduce NO_2 can be provided enzymatically by nitrate reductase (Rockel *et al.*, 2002; Stohr *et al.*, 2001; Yamasaki *et al.*, 1999) or the mitochondrial electron transport system (Planchet *et al.*, 2005), or non-enzymatically under reducing conditions and low pH in the apoplast (Bethke *et al.*, 2004). The oxidative pathway is more poorly characterized because no enzymes have yet been identified for any of the substrates mentioned (Corpas *et al.*, 2009*b*; Rumer *et al.*, 2009; Tun *et al.*, 2006). The participation of enzymatic and non-enzymatic NO sources in plant physiological processes has been extensively documented and discussed (Moreau *et al.*, 2010; Yu *et al.*, 2014).

In plants, NO production increases under several stresses and altered physiological conditions (Lamattina *et al.*, 2003). The first biotic process where increased NO production was detected was the stress generated during plant–pathogen interactions (Delledonne *et al.*, 1998; Durner *et al.*, 1998). Abiotic stresses such as drought, high salinity, cold stress, heat shock, and ultraviolet (UV) exposure also trigger NO production in plants (Beligni *et al.*, 2002; Beligni and Lamattina, 1999; García-Mata and Lamattina, 2001; Graziano and Lamattina, 2007*b*; Tossi *et al.*, 2011; Tossi *et al.*, 2012; Uchida *et al.*, 2002; Zhao *et al.*, 2004; Zhao *et al.*, 2007; Zhao *et al.*, 2009). Accumulating evidence supports the fact that NO improves a plant's antioxidant capacity to counteract the oxidative environment generated by ROS derived from stress processes, hence contributing to a general plant cell redox homeostasis. NO reduces H_2O_2 levels, ROS accumulation, and cell damage during salt stress (Bai *et al.*, 2011), UV exposure (Tossi *et al.*, 2011) and photo-oxidative stress (Beligni and Lamattina, 2002).

Thiol modification and S-nitrosylation

Thiol-containing proteins can undergo a range of oxidative post-translational modifications (PTMs) by ROS and RNS under certain conditions, from the reversible formation of S-nitrosothiol (SNO), sulfenic acid (SOH), disulfide, or sulfinic acid (SO_2^-), to the irreversible formation of sulfonic acid (SO_3^-). Evidence suggests that thiol oxidation plays a key role in the cell sensing an unbalanced redox status (Groitl and Jakob, 2014). The best-characterized global redox sensor is the bacterial transcription factor OxyR, which activates multiple genes to prevent damage due to oxidative and nitrosative stresses. The inactive form of OxyR possesses reduced thiols and acts as a redox switch interrupter, activated via the oxidation of Cys residues in its sequence (Zheng *et al.*, 1998). In response to H_2O_2 or nitrosative stress, OxyR becomes oxidized, altering its binding capacity to DNA and promoting gene activation. This oxidation involves the formation of a disulfide, SNO, SOH, and SO_2^-; these PTMs of OxyR Cys residues have been described *in vivo* (Kim *et al.*, 2002; Seth *et al.*, 2012).The relevance of the NO-mediated S-nitrosylation of Cys residues in proteins has been studied in detail. A wide variety of proteins that are S-nitrosylated in plants are associated with the cytoskeleton, primary and secondary metabolism, transcription factors, receptors, and redox-related proteins (Fares *et al.*, 2011; Lindermayr *et al.*, 2005; Romero-Puertas *et al.*, 2008). *In vivo* S-nitrosylation of catalase and glycolate oxidase have been identified in *Pisum sativum* (pea) leaf peroxisomes treated with cadmium and the herbicide 2,4-dichlorophenoxyacetic acid (Ortega-Galisteo *et al.*, 2012). In another study, 2,4-dichlorophenoxyacetic acid promoted disturbance of the actin cytoskeleton structure by actin S-nitrosylation, affecting polymerization and consequently the dynamic of the peroxisomes (Rodriguez-Serrano *et al.*, 2014). NADPH oxidase activity is inhibited by S-nitrosylation, limiting cell death development during the

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hypersensitive response (Yun *et al.*, 2011). Moreover, the regulation of hormone responses by S-nitrosylation was shown for the auxin receptor transport inhibitor response 1 (TIR1). S-nitrosylation of TIR1 enhances the auxin response, facilitating the degradation of auxin repressors and the activation of auxin-response genes (Terrile *et al.*, 2012).

Thiol-based regulation of proteins is linked to changes in cell redox homeostasis. This is relevant for plant cells and numerous key players involved in thiol modification are present. Thioredoxins (TRXs) are ubiquitous small proteins that catalyse the reduction of thiol-disulfide in target proteins through the reversible oxidation of its redox active motif Cys(x)2Cys to a disulfide. A wide range of genes encoding Trxs has been found in plants and classified according to their primary structure and subcellular localization (Gelhaye *et al.*, 2005; Meyer *et al.*, 2005; Meyer *et al.*, 2008). The high diversity of TRXs found in plants compared to mammals, bacteria and yeasts, which only possess two isoforms (Vlamis-Gardikas and Holmgren, 2002), highlights the fine-tuned control and the putative specificity of Cys reduction in plant proteins. This is probably related to the sessile nature of plants and the variety of stresses they confront in their habitats.

An as yet unsolved and intriguing point is the elucidation of putative substrate specificity for the different plant TRXs. Reduction of oxidized Trxs involves the activity of NADPH-dependent thioredoxin reductase (NTR) in the cytosol and mitochondria, thus determining the protein thiol–disulfide status, a primary factor in sensing the cell's redox status. *Arabidopsis* contains three NTR proteins: NTRA and NTRB in the cytosol and mitochondria, and NTRC in plastids (Meyer *et al.*, 2005). The NTR-TRX system (NTS) contributes to controlling the reduced or oxidized status of Cys residues in proteins and the reduced and oxidized TRX pool in the cell. The NTS is able to cleave reversible oxidations including S-nitrosothiols (Nikitovic and Holmgren, 1996). Thus, protein denitrosylation through the NTS emerged as a relevant PTM mechanism in cell signal transduction a few years ago. Benhar *et al.* (2008) have shown that TRX2 denitrosylates caspase 3, activating the caspase cascade and leading to apoptosis in human lymphocytes. In plants, S-nitrosylation of the regulator NPR1 induces the formation of inactive oligomers. Plant TRXs catalyse the salicylic acid–induced monomerization of NPR1 in the cytoplasm, resulting in nuclear translocation of active NPR1 monomers (Tada *et al.*, 2008). Recently, *Arabidopsis* TRXh5 was identified as a potent protein-SNO reductase in the plant immune response. TRXh5 is strongly induced by exogenous application of salicylic acid. Moreover, TRXh5 can discriminate between protein-SNO substrates, providing specificity and reversibility during salicylic acid–dependent plant defence signalling (Kneeshaw *et al.*, 2014). Another redox control pathway is the glutaredoxin system, responsible for disulfide reduction and deglutathionylation of target proteins (Meyer *et al.*, 2008). Nevertheless, the role of glutaredoxin system in denitrosylation still needs to be proved.

Tyrosine nitration

NO can rapidly react with O_2^-, yielding peroxynitrite ($ONOO^-$). This potent RNS can nitrate lipids, nucleic acids, aromatic rings, and tyrosine residues in proteins (Corpas *et al.*, 2009*a*; Stamler *et al.*, 1992). Tyrosine nitration is the addition of a NO_2 group to one of the ortho carbons of the aromatic ring of tyrosine. It is considered a selective PTM that can regulate enzyme activity and, in some cases, prevent or promote tyrosine phosphorylation (Kong *et al.*, 1996; Stasi *et al.*, 1999). At present, tyrosine nitration is considered a selective but irreversible process and is associated with pathological nitrosative stress conditions (Ischiropoulos, 2003). Proteomic studies have identified many proteins as targets of tyrosine nitration in plants (Cecconi *et al.*, 2009; Chaki *et al.*, 2009; Lozano-Juste *et al.*, 2011; Saito *et al.*, 2006). The first study of tyrosine nitration in plants was performed in nitrite reductase antisense tobacco plants where nitrated cyclophilin and 14-3-3 proteins were identified (Morot-Gaudry-Talarmain *et al.*, 2002). Saito *et al.*, (2006) reported nitrated proteins of 25 kDa and 50 kDa in BY-2 cells infected with an elicitin secreted by the pathogen *Phytophthora infestans*. Later studies demonstrated that *Arabidopsis* peroxiredoxin II E, involved in peroxynitrite detoxification, is inhibited by S-nitrosylation during plant defence responses. Peroxiredoxin inhibition contributes to an increasing level of tyrosine-nitrated proteins in *Arabidopsis* during infection (Romero-Puertas *et al.*, 2007). Furthermore, increased levels of nitrated proteins were found in salt-stressed olive leaves (Valderrama *et al.*, 2007). β-carbonic anhydrase, involved in the supply of CO_2 for Rubisco activity, is a target of tyrosine nitration under high temperature stress in sunflower, resulting in an inhibition of its activity and thus affecting photosynthesis (Chaki *et al.*, 2013). In another study, nitration of NADH-dependent hydroxypyruvate reductase provoked a loss of function in peroxisomes (Corpas *et al.*, 2013).

Metal nitrosyl and oxidation of zinc-thiolate complexes

NO can form a radical adduct with transition metal ions. The best studied are the complexes within the haem group of proteins and the iron–sulfur centres of proteins that result in the modification of protein activities like haemoglobin, catalase, and aconitase (Arnold *et al.*, 1977; Brown, 1995; Clark *et al.*, 2000; Doyle and Hoekstra, 1981; Navarre *et al.*, 2000). Metallothioneins, proteins containing zinc thiolate clusters, are also targets of NO, triggering intracytoplasmic zinc release (Aravindakumar *et al.*, 1999; Croix *et al.*, 2002). Remarkably, nitrosative stress can also release zinc from zinc–sulfur clusters in proteins like zinc finger transcription factors, and thus selectively affect gene expression (Kröncke and Carlberg, 2000).

A case study: the NO-mediated regulation of cytosolic ascorbate peroxidase in stress and developmental physiology

APXs are haem-containing enzymes that catalyse H_2O_2 reduction using ASC as an electron donor. They are a family of isozymes with distinct characteristics localized in different plant cell compartments: cytoplasm (cytosolic ascorbate peroxidase, cAPX), chloroplast (stromal and tilacoidal),

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peroxisomes (membrane-bound in peroxisomes and glyoxysomes), and mitochondria (membrane-bound) (Shigeoka *et al.*, 2002). APXs, together with the enzymes involved in the GSH/ASC cycle, are essential elements in the control of redox metabolism in plants (Foyer and Noctor, 2011). APX activity is modulated under various environmental stresses to control the spread and damage capacity of oxidative stress. In addition, APX also participates in physiological and developmental responses through precise localized concentration of the signal molecule H_2O_2. Regulation of APX activity contributes to the control of programmed cell death (PCD) (de Pinto *et al.*, 2006; de Pinto *et al.*, 2013), as well as regulating stress induced by high light (Karpinski *et al.*, 1997) drought (Mittler and Zilinskas, 1994) and high temperature (Panchuk *et al.*, 2002). APX activity is involved in seed germination (Arrigoni *et al.*, 1992; Bai *et al.*, 2011), leaf senescence (Panchuk *et al.*, 2005), nodule development (Keyster *et al.*, 2011), and lateral root (LR) formation (Correa-Aragunde *et al.*, 2013). Overexpressing different isoforms of APX in plants increases tolerance to heat (Shi *et al.*, 2001), chilling (Wang *et al.*, 2005), salt stress, and water deficit (Badawi *et al.*, 2004), and alleviates photo-oxidative stress induced by the herbicide Paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride) (Murgia *et al.*, 2004; Wang *et al.*, 1999). Mutations affecting APX expression increase sensitivity to diverse oxidative stresses (Davletova *et al.*, 2005; Miller *et al.*, 2007; Rossel *et al.*, 2006).

Multiple post-translational redox regulation of cAPX occurs in plants. A proteomic approach showed that cAPX is a target of TRX (Fig. 1; Marchand *et al.*, 2004; Yamazaki *et al.*, 2004). In addition, incubation of cAPX with TRXh drastically inhibits its activity *in vitro*; suggesting that, in the active form of cAPX, at least one Cys is reversibly oxidized (Gelhaye *et al.*, 2006). The oxidized Cys that can be reversibly reduced by TRX in a reversible way might be an SOH or SNO (Fig. 1). Treatment of APX with the reductants dithiothreitol and GSH also inhibits its activity (Gelhaye *et al.*, 2006). Carbonylation of residues in cAPX occurs during *Antiaris toxicaria* seed desiccation, causing an irreversible inhibition of cAPX activity (Fig. 1; Bai *et al.*, 2011). Several NO-mediated PTMs have been reported to influence APX activity (Fig. 1). Inhibition of tobacco APX activity was studied by the reversible binding of NO to the haem prosthetic group (Clark *et al.*, 2000). Moreover, studies using a peroxynitrite donor showed that another potential redox modification in cAPX is nitration of tyrosine residues in *Arabidopsis* (Lozano-Juste *et al.*, 2011). Nitrated cAPX was found in roots but not in shoots of citrus plants subjected to salinity stress (Tanou *et al.*, 2012). This modification occurs in Tyr5 and Tyr235 of pea cAPX, causing an irreversible inhibition of APX activity (Begara-Morales *et al.*, 2013). Figure 1 summarizes the richness of the PTMs altering the enzymatic activity of cAPX, providing an elegant way to manage the H_2O_2 concentration in plant cells.

Contradictory results have been reported on the effect of S-nitrosylation on cAPX activity (Fig. 1). This PTM of cAPX was first found to occur in plants in large-scale proteomic analysis (Fares *et al.*, 2011). *In vivo* S-nitrosylated cAPX was found in seeds of *A. toxicaria* and shown to prevent carbonylation of the protein. The authors speculate that S-nitrosylation of cAPX enhances its activity during seed germination (Bai *et al.*, 2011). In agreement with this study, S-nitrosylation was found to stimulate cAPX activity in salt-stressed pea plants (Begara-Morales *et al.*, 2013). Cys32 was found to be the main target of S-nitrosylation (Begara-Morales *et al.*, 2013; Fares *et al.*, 2011). Cys32 is present in 100% of the cAPX described so far and it is part of the pocket that binds ASC (Correa-Aragunde *et al.*, 2013; Gelhaye *et al.*, 2006). Mutation of Cys32 in pea cAPX resulted in the loss of two-thirds of APX activity (Mandelman *et al.*, 1998).

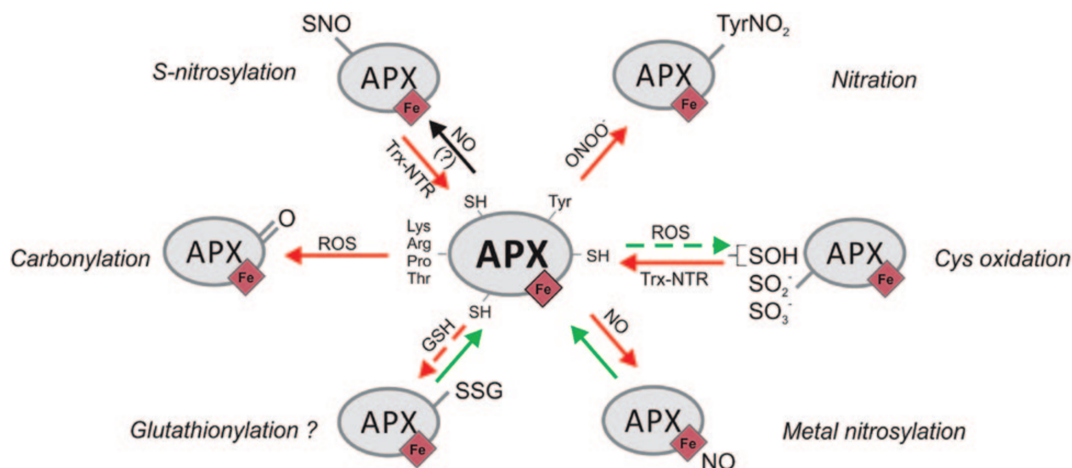

Fig. 1. Redox PTMs of cAPX result in a sensitive menu of redox-based regulation of its enzymatic activity. Excessive ROS levels cause carbonylation of residues in APX (Bai *et al.*, 2011). NO nitrosylates Cys32 residue (Bai *et al.*, 2011; Begara-Morales *et al.*, 2013; Correa-Aragunde *et al.*, 2013; de Pinto *et al.*, 2013; Fares *et al.*, 2011), or form metal adducts with the iron of the haem prosthetic group (Clark *et al.*, 2000). Peroxinitrite ($ONOO^-$) nitrate Tyr5 and Tyr235 residues in pea cytosolic APX (Begara-Morales *et al.*, 2013). Some oxidative forms of Cys residues in APX are reversible and can be reduced by the thioredoxin-NADPH–thioredoxin reductase (Trx-NTR) system (Gelhaye *et al.*, 2006; Marchand *et al.*, 2004; Yamazaki *et al.*, 2004). GSH may glutathionylate Cys residues, inhibiting APX activity (Gelhaye *et al.*, 2006). Red and green arrows mean inhibition and activation of APX activity, respectively. The black arrow and question mark indicate contradictory data about the way that S-nitrosylation affects APX activity. While some authors indicate that APX is activated by S-nitrosylation (Bai *et al.*, 2011, Begara-Morales *et al.*, 2013; Correa-Aragunde *et al.*, 2013), another study showed that it is inhibited (de Pinto *et al.*, 2013). Dashed arrows indicate inferred redox PTMs that were not experimentally confirmed.

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S-nitrosylation of cAPX also occurs under heat shock conditions during PCD in tobacco BY2 cells (Fig. 2; de Pinto *et al.*, 2013). In this experimental model, the authors describe the inhibition of enzyme activity by S-nitrosylation, ubiquitination, and degradation of cAPX under either heat stress or H_2O_2 treatment, leading to PCD (Fig. 2; de Pinto *et al.*, 2013). Figure 2 represents an alternative role for PTMs of cAPX in strong oxidative stresses (for example, heat, desiccation, salt stress, and H_2O_2 treatment) that lead to PCD. Biotic and abiotic stress induces ROS production by mainly NADPH oxidase (Miller *et al.*, 2009; Sagi and Fluhr, 2006). An acute ROS burst induces NO and other RNS formation. Simultaneous carbonylation, tyrosine nitration, and S-nitrosylation of cAPX would irreversibly affect its activity (Bai *et al.*, 2011; de Pinto *et al.*, 2013; Tanou *et al.*, 2012), inducing polyubiquitination and finally degradation by the proteasome (de Pinto *et al.*, 2013).

The link between redox balance and hormone signalling: S-nitrosylation of cytosolic APX1 during auxin-mediated root development

The participation of ROS and RNS in auxin signalling in root growth and development has been extensively studied. Auxin induces ROS production during gravitropic bending in roots in a pathway that involves the activity of phosphatidylinositol 3-kinase (Joo *et al.*, 2001; Joo *et al.*, 2005). Auxin also promotes H_2O_2 production during LR development (Fig. 3; Correa-Aragunde *et al.*, 2013; Ma *et al.*, 2014). Because of the auxin-mediated H_2O_2 increase, enzymes and molecules involved in the antioxidant system are activated to keep the ROS concentration under control. Genetic evidence supports a role for NTR and GSH metabolism in auxin signalling. The triple mutant *ntra ntrb cad2* (encoding the first enzyme involved in GSH biosynthesis) shows a loss of apical dominance, altered vasculature, and reduced LR number, all phenotypes regulated by auxin (Bashandy *et al.*, 2010).

NO plays an important role during LR and adventitious root formation in *Arabidopsis*, *Solanum lycopersicum* (tomato), *Cucumis sativus* (cucumber), *Zea mays* (maize) and *Oryza sativa* (rice) (Chen *et al.*, 2012; Correa-Aragunde *et al.*, 2004; Flores *et al.*, 2008; Mendez-Bravo *et al.*, 2010; Pagnussat *et al.*, 2002). The study of S-nitrosylated proteins during LR formation in *Arabidopsis* showed that cAPX1 is denitrosylated by auxin treatment (Fig. 3; Correa-Aragunde *et al.*, 2013). When assayed, cAPX activity decreased in roots treated with auxin. Because TRXs and reductases are cellular systems involved in denitrosylation, it was speculated that cAPX1 could be denitrosylated by these systems and, as stated above, Bashandy *et al.* (2010) demonstrated the involvement of NTS and GSH in auxin signalling in *Arabidopsis*. The inhibitor of the NTR activity, auranofin, increases the level of S-nitrosylated cAPX forms coincident with a higher APX

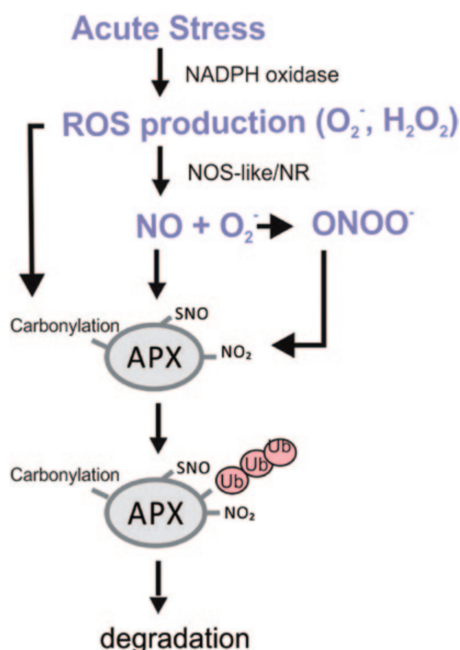

Fig. 2. ROS and RNS generated by acute physiological stresses (salt, drought, heat shock, and PCD) lead to APX degradation. APX is a target of several redox modifications (nitrosylation, nitration, and carbonylation) that affect its activity (Bai *et al.*, 2011; de Pinto *et al.*, 2013; Tanou *et al.*, 2012). These redox-mediated PTMs may be signals determining the ubiquitination of APX and its consequent degradation via proteasomes. NR, nitrate reductase. (This figure is available in colour at *JXB* online).

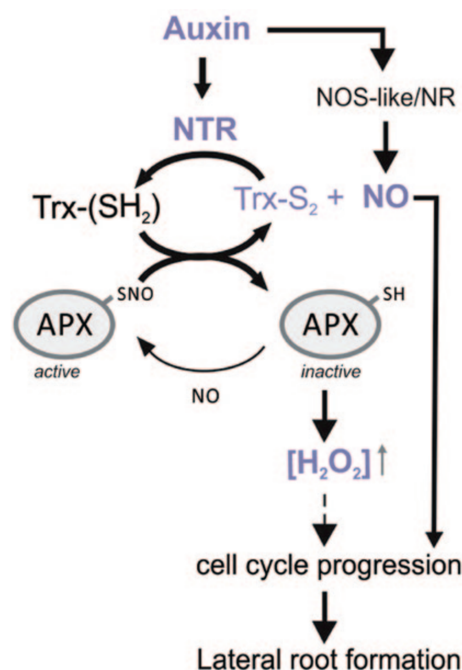

Fig. 3. Schematic model representing the redox regulation of APX1 and its contribution to auxin-induced LR formation. Auxin induces NTR activity and the increase of a reduced Trx (Trx-SH_2) pool contributes to the denitrosylation of proteins. Reduced Trx can denitrosylate APX1 and cause its partial inactivation (Correa-Aragunde *et al.*, 2013; Gelhaye *et al.*, 2006). Denitrosylation of S-nitrosothiols by the NTR-Trx system releases NO from proteins. Auxin also induces NO production in roots through nitrate reductase (NR)- and NOS-like activities. Inactivation of APX1 by denitrosylation results in H_2O_2 accumulation in roots, required for auxin-induced regulation of root growth through the activation of cell cycle progression and LR formation. (This figure is available in colour at *JXB* online).

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activity in roots (Correa-Aragunde *et al.*, 2013). The involvement of cAPX1 in LR formation was demonstrated using *Arabidopsis apx1* mutants, which display a higher concentration of H_2O_2, shorter roots, and fewer LRs than the wild type. Interestingly, *Arabidopsis apx1* mutants are less sensitive to auxin treatment than the wild type (Correa-Aragunde *et al.*, 2013).

Figure 3 is a model that represents the crossroad of information between auxin and NO signalling in the control of redox balance and APX1 activity in the determination of root architecture in *Arabidopsis*. Auxin induces NTR activity (Bashandy *et al.*, 2010) and NO production in roots by nitrate reductase and NOS-like activities (Flores *et al.*, 2008; Kolbert *et al.*, 2007; Wang *et al.*, 2010*a*, *b*). NTR activity promotes denitrosylation of APX1 by TRX. Denitrosylation of APX1 partially inhibits its enzymatic activity, leading to an increase of H_2O_2 levels that acts as a signal together with NO for the promotion of LR formation.

Concluding remarks

Oxidative and nitrosative stresses refer, respectively, to the imbalance generated from an excess of ROS and RNS over the cell's capacity to maintain the cell redox homeostasis. At the same time, precise cell signalling pathways are activated in parallel by specific ROS and RNS compounds. The research of target molecules of ROS and RNS in cell signalling is a matter of intense study. ROS-mediated activation of signalling pathways has been studied for years. However, less well understood are the signals activated by RNS and the specific molecules and chemical basis underpinning the PTMs of target proteins that lead to the fine regulation of redox mechanisms in cell physiology.

The antioxidant capacity of NO to influence cell redox signalling and physiology relies on the following conceptual frame. Once any alteration of intra- or extracellular homeostasis occurs, including those provoked by growth and developmental processes, there is a redox imbalance that alters membrane function and results in an increased production of ROS. Cells immediately activate a strategy to reach a new redox equilibrium. This response involves the production of NO and GSH and the consequent formation of S-nitrosoglutathione, which can sequester iron in low molecular weight compounds named mono- and dinitrosyl iron complexes (Graziano and Lamattina, 2007*a*; Vanin, 2009). This rapid cell response mitigates the iron-induced propagation of peroxidative processes in lipids, proteins, and nucleic acids. As stated above, the increase of NO and S-nitrosoglutathione also results in an augmented S-nitrosylation of Cys residues in proteins, which is an oxidative but reversible process that can be counterbalanced by cellular reductases like TRXs and S-nitrosoglutathione reductases. Even if the primary consequence of S-nitrosylation is the oxidation of proteins, besides its physiological significance as a signalling PTM, it can protect Cys residues from subsequent strong and irreversible oxidations due to higher ROS concentrations.

Here, we illustrate a variety of redox-regulated PTMs occurring in an enzyme that is only present in plant cells, APX. It is a challenge to understand how cells sense a redox imbalance generated by exogenous and/or endogenous stimuli and discriminate, through the regulation of the antioxidant response, between actions determining the cell's growth and developmental from those involved in stress physiology metabolism. From a more general perspective, we highlight the close interaction occurring between ROS and RNS in determining the PTMs of proteins mediated by redox changes. The thiol groups and tyrosine residues of target proteins are fine sensors of acute or slight cell redox imbalances, being able of generating a myriad of reversible and irreversible changes in the activities and cellular functions of proteins, in a direction that contributes to the complex mechanism controlling cell homeostasis.

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