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

Natalia Correa-Aragunde, Noelia Foresi and Lorenzo Lamattina*

Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CC 1245, 7600 Mar del Plata, Argentina

* To whom correspondence should be addressed. E-mail: lolama@mdp.edu.ar

Received 13 November 2014; Revised 4 November 2015; Accepted 5 February 2015

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 O2 to form the free radical anion superoxide (O2−). Plasma membrane-NADPH oxidase also generates O2− (Sagi and Fluhr, 2006). This oxidant agent is highly reactive and a substrate for the generation of other ROS. Superoxide dismutase dismutates O2− into hydrogen peroxide (H2O2). In turn, H2O2 generates hydroxyl radicals (OH·) in combination with metals in a process called the Fenton reaction. These extremely reactive molecules (O2−, H2O2, OH·) 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, mono-dehydroascorbate; 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.

© The Author 2015. Published by Oxford University Press on behalf of the Society for Experimental Biology. All rights reserved.

For permissions, please email: journals.permissions@oup.com
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 H₂O₂ levels in a series of reactions called the ascorbate–glutathione cycle. For instance, the enzyme ascorbate peroxidase (APX) reduces H₂O₂ to H₂O with ASC as an electron donor [2ASC + H₂O₂ → 2 monodehydroascorbate (MDA) + 2H₂O]. Ascorbate is regenerated from MDA by the activity of MDA reductase (NADH + 2MDA ⇌ 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 → 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⁺) with an unpaired electron in the 2p π orbital, and the nitronium cation (NO²⁺). 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 (NO₂⁻) 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 NO₂ 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., 2009b; 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; Garcia-Mata and Lamattina, 2001; Graziano and Lamattina, 2007b; 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 H₂O₂ 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 (SO₂⁻), to the irreversible formation of sulfonic acid (SO₃⁻). Evidence suggests that thiol oxidation plays a key role in the cell sensing an unbalanced redox status (Groil 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 H₂O₂ 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 SO₂⁻; 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 glycocalyx 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
Nitric oxide is a ubiquitous signal for maintaining redox balance in plant cells

hypothesis 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-disulphide in target proteins through the reversible oxidation of its redox active motif Cys(x)2Cys to a disulphide. A wide range of genes encoding TRx 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–disulphide 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 reduct control pathway is the glutaredoxin system, responsible for disulphide 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

Nitric oxide 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., 2009a; 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 elicitor 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–sulphur 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 cystolic ascorbate peroxidase in stress and developmental physiology

APXs are haem-containing enzymes that catalyse H$_2$O$_2$ reduction using ASC as an electron donor. They are a family of isozymes with distinct characteristics localized in different plant cell compartments: cytoplasm (cystolic ascorbate peroxidase, cAPX), chloroplast (stromal and tilacoidal),
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 H₂O₂. 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 (Keister 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 (Davelletova 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 APX 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 H₂O₂ 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., 2011). Peroxynitrite (ONOO⁻) nitrates 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.](https://academic.oup.com/jxb/article-abstract/66/10/2913/533440 by guest on 28 March 2019)
Nitric oxide is a ubiquitous signal for maintaining redox balance in plant cells

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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ production during LR development (Fig. 3; Correa-Aragunde et al., 2013; Ma et al., 2014). Because of the auxin-mediated H$_2$O$_2$ 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) 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$_2$O$_2$ 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.)
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$_2$O$_2$, 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., 2010a, b). NTR activity promotes denitrosylation of APX1 by TRX. Denitrosylation of APX1 partially inhibits its enzymatic activity, leading to an increase of H$_2$O$_2$ 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, 2007a; 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.

References


glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *The Plant Cell* 12, 97–110.


