The thioredoxin/peroxiredoxin/sulfiredoxin system: current overview on its redox function in plants and regulation by reactive oxygen and nitrogen species

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Abstract

In plants, the presence of thioredoxin (Trx), peroxiredoxin (Prx), and sulfiredoxin (Srx) has been reported as a component of a redox system involved in the control of dithiol–disulfide exchanges of target proteins, which modulate redox signalling during development and stress adaptation. Plant thiols, and specifically redox state and regulation of thiol groups of cysteinyl residues in proteins and transcription factors, are emerging as key components in the plant response to almost all stress conditions. They function in both redox sensing and signal transduction pathways. Scarce information exists on the transcriptional regulation of genes encoding Trx/Prx and on the transcriptional and post-transcriptional control exercised by these proteins on their putative targets. As another point of control, post-translational regulation of the proteins, such as S-nitrosylation and S-oxidation, is of increasing interest for its effect on protein structure and function. Special attention is given to the involvement of the Trx/Prx/Srx system and its redox state in plant signalling under stress, more specifically under abiotic stress conditions, as an important cue that influences plant yield and growth. This review focuses on the regulation of Trx and Prx through cysteine S-oxidation and/or S-nitrosylation, which affects their functionality. Some examples of redox regulation of transcription factors and Trx- and Prx-related genes are also presented.

Key words: Peroxiredoxin, redox gene regulation, signalling, S-nitrosylation, S-oxidation, sulfiredoxin, thioredoxin.

Introduction

Plant cells generate reactive oxygen (ROS) and nitrogen (RNS) species involved in the general metabolism. These reactive species can be harmful for cellular components due to their chemistry, but they are also involved in cellular signalling to promote defence against environmental stress situations (Marti et al., 2011; Foyer and Noctor, 2013; Lázaro et al., 2013). Consequently, plants have evolved a battery of redundant and elaborated mechanisms, including metabolites and enzymes, that are responsible for controlling ROS/RNS levels involved in oxidative and nitrosative insults responding to environmental cues (Noctor et al., 2014; Talbi et al., 2015).

Owing to the reactivity of their thiol groups, some protein cysteine residues are highly sensitive to oxidation by these reactive molecules, which may perturb cellular homeostasis. Thiol reduction is controlled mainly by the thioredoxin (Trx)/peroxiredoxin (Prx) and glutathione (GSH) systems, which respond to stress situations to regulate redox homeostasis. Thioredoxins are small proteins (around 12kDa) containing two cysteines in the redox active centre present in all life...
forms. They regulate the function of target proteins through oxidoreductase activity. Trx couples with Trx-dependent peroxidases (Prxs) to scavenge hydrogen peroxide (H$_2$O$_2$) and peroxinitrite (König et al., 2002; Balmer et al., 2004; Barranco-Medina et al., 2007). In addition, Trx does not simply act as a scavenger of ROS but also as an important regulator of the oxidative stress response through protein–protein interactions (Martí et al., 2011; Zhang et al., 2011). Changes in thiol status of proteins probably play a role in redox signalling under biotic and abiotic stress. Specifically, modifications in thiol-containing enzymes are reported to have an impact on their structure and functionality, and thus in the perception and control of ROS and RNS changes occurring under stress situations (Spoel and Loake, 2011).

An interesting aspect in cell biology is the control that the Trx/Prx system can exert on the transcriptional system, a signalling process occurring in several pathological and environmental stress conditions. Trxs can set the required redox state of the transcription factors (TFs) to be bound to the promoter region of DNA. Prxs, through the H$_2$O$_2$ control, may have an impact on the redox-mediated regulation of transcription (Liu et al., 2013). These TFs couple environmental stress to gene expression, and, for some, overexpression has been reported to increase plant stress tolerance (Hu et al., 2006).

In this review, we summarize some representative examples of the regulation of Trx and Prx through cysteine S-oxidation and/or S-nitrosylation by affecting their functionality in a particular cellular process and/or stress situation. A comparative analysis of plant, animal, and yeast systems is presented. In addition, we consider some examples linking gene and/or TF regulation to redox changes under stress situations. Finally, we describe the emerging knowledge on the transcriptional regulation of Trx and Prx genes.

**ROS and RNS drive conformational changes in the Trx/Prx system: functional significance**

ROS generated as by-products of cellular metabolism are known to produce reversible oxidative modifications in proteins by altering their activity or increasing their susceptibility to aggregation and degradation. Interestingly, many of the key players involved in the defence against oxidative stress are redox-sensitive proteins containing cysteine (Cys), methionine (Met), or histidine (His) in their amino acid sequence or metals in their active centres. The redox state of these residues is directly involved in the protein structure and functionality (Couturier et al., 2013). Cys is one of the best-conserved amino acids in proteins, although it accounts for only 2% of amino acid content in cells (Lindahl et al., 2011). Cys residues perform important functions in the cell by playing structural roles as metal ligands and because they are susceptible to several post-translational modifications (PTMs). Cysteine SH can also react with neighbouring SH groups to form a disulfide bridge. While as free amino acid the –SH group of Cys has a pK$_{a}$ of about 8.3, in thiol oxidoreductase proteins, like Trxs and glutaredoxins, reactive Cys possess a lower pK$_{a}$ (3–7). This pK$_{a}$ is influenced by the surrounding micro-environment (Mailloux et al., 2014). The presence of adjacent positively charged amino acids like lysine in glutaredoxins (Stroher and Millar, 2012) or the close proximity of a His residue in sulliredoxin (Srx) (Iglesias-Baena et al., 2010) can decrease the pK$_{a}$ of the Cys. Thus, at a physiological pH, these Cys residues will appear predominantly as more-reactive thiolates and facilitate different oxidation states in response to redox signals.

H$_2$O$_2$ is the most relevant ROS for mediating oxidative reactions due to its reactivity with cysteine, its prolonged half-life relative to other ROS, and its capacity to diffuse through membranes. During redox signalling, H$_2$O$_2$ oxidizes the thiolate anion of protein Cys residues to the sulfenic form (Cys–SOH) (Fig. 1A). The reversibility of the process can serve as a signal transduction mechanism, ensuring transient signalling and avoiding irreversible overoxidation of the thiol (Brandes et al., 2009). The sulfenic form can also react with another thiolate to form an intra- or intermolecular disulfide bond. The sulfenic form is estimated to be formed in the nanomolar range of H$_2$O$_2$, whereas higher levels oxidize thiolates to a sulfonic (SO$_2$H) or sulfonic (SO$_3$H) species, which can be irreversible. Control of H$_2$O$_2$ levels must, therefore, be tightly exerted by the protective antioxidant system to avoid overoxidation of functional proteins. During a stress situation, changes in the Cys redox status trigger signalling pathways, influencing the response to the biotic and abiotic stress responsible for the oxidative situation. However, these redox signalling mechanisms are dependent on the extreme specificity for the appropriate substrates.

The modification of Cys by nitric oxide (NO) forming S-nitrosothiols occurs through its reaction with thyl radicals formed from one electron oxidation of thiolates via NO$_2$. Additionally, NO$_3$ can react with NO to form N$_2$O$_3$, which can combine with cysteine thiolate to form the S-nitrosothiol. Another possibility is the transfer to a free thiol group of a haem-bound NO or the trans-nitrosylation (transfer of NO) from an S-nitrosylated residue to another thiolate or to GSH to form GSNO, the physiological NO transporter and reservoir. This GSNO contributes to protein S-nitrosylation and
S-glutathionylation, which is thought to be part of the signalling transduction (Astier and Lindermayr, 2012) (Fig. 1B).

Thiol modification of Trx mediated by \( H_2O_2 \)

Trxs contain a conserved active site, WCG/PPC, which is essential for the redox regulation of specific target proteins. In plants, there are at least 10 families of Trxs, with more than 40 members present in almost all the cellular compartments (see Gelhaye et al., 2005; Marti et al., 2009; Meyer et al., 2012; Traverso et al., 2013). The diversity of isoforms seems to support plants with an additional antioxidation system, compared with mammals, where only two types of Trx have been described: Trx1 and Trx2, in the cytosol and mitochondria, respectively (Lillig and Holmgren, 2007).

As a disulfide reductase, Trx catalyses the reduction of disulfides to dithiol in target proteins at even faster rates than dithiothreitol or GSH. The redox mechanism is based on the reversible oxidation of two Trx cysteine thiol groups to a disulfide, with the transfer of two electrons and two protons (Holmgren, 1995). However, a redox-dependent switch in protein structure and function in response to environmental stress has been described for several Trxs (Chi et al., 2013).

Arabidopsis thaliana AtTDX (Trx-like protein) composed of two domains, a Trx motif and a tetratricopeptide-repeat motif (Kim et al., 2010), and NTRC (NADPH-Trx reductase, type c), containing an N-terminal thioredoxin reductase (TR) and a C-terminal Trx domain (Pérez-Ruiz et al., 2006), in addition to AtTrx-h3, AtTrx f, and AtTrx m, have all been shown to function as a disulfide reductase, foldase chaperone, and holdase chaperone (Park et al., 2009; Sanz-Barrio et al., 2012; Chae et al., 2013). All presented disulfide reductase and foldase chaperone activities in their low-molecular-weight form, while the holdase activity was presented by the high-molecular-weight complexes. It has been reported that oligomerization status is regulated by heat shock and ROS concentration for AtTDX, which provokes the change to oligomeric complexes and a functional switch to holdase chaperone (Lee et al., 2009). Through this activity, and as a protecting mechanism of protein self-aggregation, overexpressing AtTDX Arabidopsis plants presented more resistance to heat-shock stress. Moreover, it seems that the Cys residues in the active site are involved in the disulfide activity, as described for Trx f and AtTrx-h3 (Park et al., 2009; Sanz-Barrio et al., 2012), but not in the holdase chaperone. However, mutation in non-active-site Cys in Trx f has also been found to display a strong influence in the disulfide reductase and chaperone foldase functions. More recently, Arabidopsis tetratricopeptide Trx-like (TTL) proteins, which are essential for salt and osmotic stress tolerance, have been shown to be possible co-chaperones that interact with Hsp90 and Hsp70 (Prasad et al., 2010). Through this chaperone-like activity, these proteins could be involved in the stress tolerance.

Thiol modification of Prx mediated by \( H_2O_2 \)

Prxs are thiol-based peroxidases involved in peroxide detoxification and signalling. More recently, it has been demonstrated that Prxs undergo multiple functions as a molecular chaperone, enzyme activator, protein binding partner and redox sensor (Caporaletti et al., 2007; Barranco-Medina et al., 2008; O’Neill et al., 2011). In Arabidopsis plants, 10 Prx genes have been classified according to their number and position of the conserved Cys, into four functional categories: 1-Cys Prx, 2-Cys Prx, Type-II Prx, and Prx Q. These are located in different compartments, such as the cytosol, mitochondria, chloroplasts, and nucleus (Rouhier and Jacquot, 2005; Barranco-Medina et al., 2007; Iglesias-Baena et al., 2010; Dietz, 2011). In mammals and humans, six different Prxs (Prx I–VI) are classified and grouped into three types (Rhee et al., 2012). Under the normal catalytic cycle, Prxs are selectively oxidized by \( H_2O_2 \) to sulfenic acid (Cys-SOH) at its peroxidatic cysteine (Cp). The Cp in 2-Cys-Prx then reacts with a resolving cysteine (Cp) located in the other subunit of the homodimer to produce an intermolecular disulfide. However, an intramolecular disulfide is formed in the atypical 2-Cys Prxs including hPrx V and plant Prx IIF (Seo et al., 2000; Barranco-Medina et al., 2007). The disulfide or sulfenic acid form of these Prxs is subsequently and specifically converted back to a reduced state by biological thiols, such as Trx or glutaredoxin (Rouhier et al., 2002; Barranco-Medina et al., 2007, 2009; Meyer et al., 2012). The Cys sulfenic form can be hyperoxidized by peroxide to form a more stable sulfenic Cys (Cys-SO2H). This form can be reduced by Srx via the formation of a phosphoryl intermediate on the sulfanyl moiety, attacked by the catalytic Cys of Srx. Finally, a thiosulfinate intermediate between Prx and Srx is formed (Biteau et al., 2003; Jönsson et al., 2008; Iglesias-Baena et al., 2010) (Fig. 2). However, a hyperoxidized form of the sulfenic form to a sulfonic Cys is irreversible to the best of our knowledge. In pea and Arabidopsis plants, Srxs are located in chloroplasts and mitochondria (Rey et al., 2007; Iglesias-Baena et al., 2010). In addition to the well-known function of chloroplastic 2-Cys Prx, the mitochondrial Srx isoform has shown a broader specificity. It is able to retroreduce the sulfenic forms of atypical plant mitochondrial PrxII F and atypical human PrxV (Iglesias-Baena et al., 2011).

Under conditions of extreme oxidative stress, increasing \( H_2O_2 \) flux leads to overoxidation of the peroxidatic Cys to sulfenic acid and enzyme inactivation. This probably acts as a dam to \( H_2O_2 \) flow or leaves sufficient time for \( H_2O_2 \) to mediate signalling (Poole and Nelson, 2008). Prx inactivation via overoxidation may be a way to accumulate \( H_2O_2 \) to allow oxidation of other redox proteins (the ‘floodgate’ hypothesis) (Wood et al., 2003). In this way, Prxs may continuously interpret and report peroxide levels by using their redox and oligomeric states. They could function as highly sensitive peroxide dosimeters that link oxidant metabolism to a variety of redox-dependent processes required for cell cycle re-entry (Phalen et al., 2006).

The different mechanisms in which sensitivity to oxidation of 2-Cys Prxs is involved in its signalling function have been reviewed (Hall et al., 2009). Two of the proposed mechanisms are related to stress response and rely on a gain of function through disulfide exchange with other sensor
proteins or on a switch to a chaperone activity. The third mechanism relies on a loss of function as peroxidase, thus allowing localized \( \text{H}_2\text{O}_2 \) build-up as described in the flood-gate model, although this mechanism is not clearly proven. Any structural change in Prxs as a consequence of oxidative stress could affect their redox state, oligomeric structure, and/or interaction with other proteins and have a significant impact on the cascade of signalling events. In pea plants, PsPrxIIIF has been shown to interact in vitro and in vivo with the mitochondrial PsTrxol (Barranco-Medina et al., 2008), and both proteins probably protect mitochondria from oxidative stress in saline conditions (Martí et al., 2011). In vitro interaction of PsPrxIIIF–PsTrxol was dependent on the catalytic residues, redox state, and conformational changes of the protein. This interaction has been observed in isolated pea mitochondria in both oxidant and reducing conditions (Barranco-Medina et al., 2008).

The acquisition of a new function of Prxs as molecular chaperone has been demonstrated in plants and animals. In plants, a functional switch from peroxidase to chaperone activity of the 1-Cys Prx and 2-Cys Prx from Chinese cabbage was induced by oxidative stress, and was accompanied by both conformational and oligomeric changes. However, another 2-Cys Prx (CPrxII) showed the highest peroxidase activity but no chaperone activity, as observed in the other two isotypes. The capacity of both Prxs to switch between roles as peroxidases and chaperones is probably involved to some extent in the response under different environment conditions (Kim et al., 2012). More recently, a collection of 2-CysPrx variants was studied to establish an unequivocal link between conformation and function (König et al., 2013). In these variants, depending on the amino acid substituting at Cys, tyrosine or phenylalanine, the peroxidase and/or chaperone functions were strongly influenced, as was the peroxidation sensibility. The authors underlined the importance of the advance in our understanding of multifunctional properties of 2-Cys Prx, which is probably involved in the observed moderate tolerance to oxidative and temperature stresses when overexpressed (An et al., 2011). In Pseudomonas, the observed functional switch seems to be regulated by an additional non-active Cys112 residue. Mutated variants with an additional Cys increased the peroxidase activity, while their chaperone activities decreased compared with that of the wild type. Thus, the additional residues seemed to induce a structural change of the protein that favoured its dual function (An et al., 2011).

Other interesting recent studies have proposed that the redox status of Prxs constitutes a rhythmic biomarker that exhibits circadian oscillations in cells from humans, mammals, and plants, most likely reflecting an endogenous rhythm in the generation of ROS (O’Neill and Reddy, 2011). In fact, in Arabidopsis plants, hyperoxidized Prx has been shown to exhibit oscillations in constant light conditions (Edgar et al., 2012). This emerging, unexplored mechanism influences its redox state.

**Thiol modification of Trx mediated by NO**

\( S \)-Nitrosylation may have a significant role as a protective reversible mechanism against irreversible oxidation of thiol groups in proteins during oxidative and nitrosative stress. Specifically, \( S \)-nitrosylation of Trx has been studied extensively in the human Trx1, which contains three structural Cys, in addition to the two active-site ones. Inconsistent results have been reported for the specificity of the GSNO-dependent nitrosylation of the three non-active-site Cys residues. Cys62 was described as \( S \)-nitrosylated from crystal structure analysis, despite being a buried residue, which after oxidation is exposed and therefore might be \( S \)-nitrosylated (Weichsel et al., 2010). However, in other studies, Cys69 or Cys73 are reported as primary sites of \( S \)-nitrosylation. Barglow et al. (2011) reported that human reduced Trx1 was nitrosylated at Cys62 faster than the oxidized Trx1 form, which presented only Cys73 as modified.
while no modification was observed in other Cys residues. Moreover, S-nitrosylation occurred only at high concentrations of GSNO (1 mM), while at biological concentrations, Trx1 was S-nitrosylated only in a single Cys (Cys62 or Cys73). The redox state of the Cys residues, the concentration of GSNO used, and the experimental conditions employed by the different groups could all be responsible for the contradictory results described. While oxidized Trx1 was reported to be S-nitrosylated in Cys73, this modification was not detectable in reduced Trx1 (Wu et al., 2010). These authors also showed a trans-nitrosylating activity for Trx1, as described previously by Hashemy and Holmgren (2008). Moreover, Prx1 was identified among 47 new Trx trans-nitrosylating target proteins, and the S-nitrosylation prevented its H$_2$O$_2$-induced overoxidation, thus protecting HeLa cells from apoptosis (Wu et al., 2010). In fact, the S-nitrosylation of Trx1 on Cys73 only occurred after the disulfide bond formation between Cys32 and Cy35, and the S-nitrosylated protein was able to trans-nitrosylate caspase-3, a main executor in the apoptotic signalling pathway. All these results point to oxidative and nitrosative alternating modifications of specific Cys residues in Trx as key regulating events in the protein functionality. As indicated previously, stress situations can regulate the redox state of the different Cys, resulting in a change in structure and in the reduction of trans-nitrosylating or denitrosylating activities of the protein.

Denitrosylation has been described as fundamental for the reversibility of the modification, and Trx1/TR are involved in this process in mammalian cells, with direct activity towards S-nitrosylated (SNO) proteins. This denitrosylating activity is carried out via the catalytic Cys32 and Cys35 (Sengupta and Holmgren, 2013). SNO-Trx1 is able to trans-nitrosylate caspase-3, thus preventing apoptosis, while reduced Trx1 denitrosylates SNO-caspase-3. The last function has also been described for mitochondrial Trx2 on stimulation of the death receptor Fas (Sun et al., 2013). Thus, SNO-protein denitrosylation/trans-nitrosylation could imply an additional function for the Trx system as a key regulating point in the apoptotic process. In fact, a decrease in trans-nitrosylation could facilitate cell death in medical disorders, while increasing S-nitrosylation of caspase-3 could lead to higher survival rates of damaged cells.

Research on the role of S-nitrosylation on Trx in plants is limited. Plant Trxnm5 has been detected as S-nitrosylated protein together with glyceraldehyde 3-phosphate dehydrogenase (also identified in Arabidopsis after nitrosylating treatments; Lindermayr et al., 2005). However, the S-nitrosylation was found only in a nitric oxide excess1 (noel) mutant of rice and not in control plants. Recently, in Brassica juncea seedlings under cold stress, Trxh was shown to be a target of S-nitrosylation, probably as a result of the NO produced (Sehrawat and Deswal, 2014).

The S-nitrosylation/denitrosylation process appears to be influenced by several NO species, redox proteins, enzymatic denitrosylase activities such as GSNO reductase (GSNOR), the Cys residue environment of the involved proteins, and the subcellular location of all of these components. The complexity can again be shown in the mechanism involving redox signals through cytosolic Trxs and GSNO in plants. Both are involved in the oligomer–monomer exchange of NPR1 (non-expressor of pathogenesis-related gene 1) on pathogen challenge in A. thaliana. Without pathogen infection, NPR1 is located in the cytosol as an inactive oligomer maintained through intermolecular disulfide bonds between solvent-exposed Cys residues. After pathogen infection, salicylic acid (SA) induces the reduction to monomers, which are translocated to the nucleus, promoting the transcriptional activation of SA-dependent defence-related genes (Mou et al., 2003). However, additional results have been reported related to the regulation of NPR1 through the opposing action of GSNO and Trx in the NO-mediated S-nitrosylation. It has been shown that GSNO S-nitrosylates NPR1 at Cys156 and may facilitate its oligomerization through disulfide linkage between some of the NPR1 monomers, preventing NPR1 translocation to the nucleus (Tada et al., 2008). Moreover, Trxh5 is required for in vivo SA-induced monomer release, probably preventing oligomerization. However, data obtained in GSNO-treated Arabidopsis protoplasts by Lindermayr et al. (2010) showed that the treatment produced S-nitrosylation of both NPR1 and TGA1 [TGACG motif binding factor 1, a basic leucine zipper (bZIP) protein]. This favoured NPR1 translocation into the nucleus and enhanced TGA1 binding activity to the promoter regions of defence-related genes. The fact that oligomerization mediated by S-nitrosylation did not inhibit NPR1 translocation implies that the mechanism of oligomer–monomer exchange regulating nuclear translocation remains unclear. Likewise, due to the possible glutathionylating effect of GSNO used in the experiments, the type of modification responsible for the increased DNA-binding activity of TGA1 is not completely elucidated. These authors even reported oligomerization induced by S-nitrosylation as a step prior to monomer accumulation compatible with the results of Tada et al. (2008). The revealed complexity of the mechanism could explain the apparently contradictory experimental data. Interestingly, the involvement of Trx as a denitrosylase in this process was recently reported by Kneeshaw et al. (2014), demonstrating that Trxh5 was able to denitrosylate TGA1 in the plant immune response. This is very interesting given the scarce knowledge about the involvement of plant Trxs in denitrosylation processes. The Trxh5/NTRA system was shown to be able to denitrosylate a variety of plant proteins in both in vitro and in vivo experiments, suggesting that Trxh5 facilitates monomerization and nuclear translocation. Moreover, the SNO-Trxh5 intermediate was detected in the process of trans-denitrosylation of Trxh5 to SNO-protein targets, using a single active-site Cys. However, the detailed mechanism is not completely elucidated. This is in agreement with the finding of Trxh3 as an S-nitrosylated protein in Arabidopsis leaves only after challenge by the pathogen Pseudomonas syringae, which probably represents a link between gene regulation and redox changes triggered by pathogens (Maldonado-Alconada et al., 2011). Moreover, the Trxh5/NTRA system has been shown to be unable to denitrosylate S-nitrosylated glyceraldehyde |
Thiol modifications of Prx mediated by NO

Besides the involvement of NO and associated $S$-nitrosylation in the above signalling processes related to Trxs, several studies have shown that Prxs from animals and plants are targets of $S$-nitrosylation under normal and stress conditions. Prx2, the most abundant Prx in mammalian neurons, is a target of $S$-nitrosylation in human Parkinson’s disease brains. The reaction of NO with two critical Cys residues (Cys51 and Cys172) (Fang et al., 2007) reduced enzyme activity and prevented its overoxidation by exogenous H$_2$O$_2$. Mammalian Prx1 is also $S$-nitrosylated, causing oligomeric structural and functional alterations and loss of peroxidase activity. In this sense, treatment with $S$-nitrosocysteine (SNO-Cys) modulated the Trx/Prx/TR system through $S$-nitrosylation of TR and Prx1. Trx-mediated regeneration of oxidized Prx1 was blocked by SNO-Cys, probably through a competition mechanism with Prx1 for the Trx system (Engelman et al., 2013).

Recently, the application in plants of exogenous NO donors like $S$-nitrosylating agents and the proteome-wide analysis upon biotic and abiotic stressors have identified a growing list of $S$-nitrosylated candidate proteins. In Arabidopsis leaves, cytosolic PrxII B and a chloroplastic PrxII E proteins that are specifically $S$-nitrosylated during the plant immune response (Lindermayr et al., 2005; Romero-Puertas et al., 2007). $S$-Nitrosylation in PrxII E was demonstrated to be a specific time-dependent response, inhibiting its peroxidase and peroxynitrite reductase activity. A model was suggested in which this PTM regulates the transduction of NO- and ROS-linked signals during infection by P. syringae, highlighting a key role for PrxII E in controlling the endogenous level of ONOO$^-$ (Romero-Puertas et al., 2007, 2008). In Brassica juncea extracts treated with GSNO, 1-Cys Prx was identified among the $S$-nitrosylated proteins, although it did not appear as differentially $S$-nitrosylated under cold stress (Abat and Deswal, 2009). Also, in wild rice and in an noel rice mutant subjected to H$_2$O$_2$-induced leaf cell death, a chloroplastic PrxII E was found as target of $S$-nitrosylation (Lin et al., 2012).

Recent evidence has shown an interplay between oxidative and nitrosative stress through the carbonylation and $S$-nitrosylation of proteins in plants subjected to salt stress (Tanou et al., 2009). The $S$-nitrosylation of specific active Cys residues leads to a conformational change that possibly prevents their carbonylation and thus the irreversible loss of protein function under salt stress. In this study, Prx is described as one of the proteins suffering carbonylation and $S$-nitrosylation, suggesting a fine regulation of protein PTMs under oxidative and nitrosative stress, which could define their abundance, function, and localization.

In the response to salt stress, the mitochondrial pea Prx was specifically $S$-nitrosylated in a time-dependent manner over a long but not a short time period, possibly responding to increased NO in part associated with mitochondria under long-term salt stress (Camejo et al., 2013). Subsequently, we have demonstrated that recombinant mitochondrial PrxII F is modified by GSNO and sodium nitroprusside dehydrate treatments resulting in a conformational change and a functional switch. Thus, the peroxidase activity of the $S$-nitrosylated PrxII F was reduced and a novel function as trans-nitrosylase activity has been reported recently when incubated with citrate synthase protein, thus preventing its thermal aggregation (Camejo et al., 2015). It would be interesting to know whether this trans-nitrosylating activity also occurs in vivo in salt-stressed plants in which mitochondrial pea Prx appeared as $S$-nitrosylated.

Redox regulation of gene transcription under stress

As a result of the increased cellular ROS production in response to environmental stress, organelle signals enter the nucleus to modulate gene expression. However, the actual sensors and second messengers of the retrograde signalling cascade are not well understood (Foyer and Noctor, 2013). The rapid increase in ROS may alert a sensor molecule that transduces the signal binding to an effector, such as the responsive elements in DNA, thus allowing gene expression. Reactive thiol proteins appear as good candidates to act as sensors for the ROS transmission of the information to the transcription system. A link between gene regulation and redox changes has been reported under the stress response (Astier et al., 2011), and the slow return to the Prx sulfenic state by Srx has been reported recently as being a crucial property for a direct or indirect oxidative stress sensor (Wood et al., 2003; Lázaro et al., 2013).

ROS-responsive genes related to Trxs and Prxs

Transcriptional profiling of Arabidopsis plants subjected to several abiotic stress conditions has identified several cis-regulatory elements associated with ROS (Allu et al., 2014). Some of these ROS-responsive elements have the G-box element, CACGTG, involved in the response to abiotic stress and hormone signalling, particularly abscisic acid (ABA). A W-box (TTGAC/T) is also recognized by WRKY (from the WRKY sequence at the N terminus), a TF induced by ROS under oxidative stress conditions (Laloi et al., 2004). Moreover, AtTRXh5 was upregulated in plants overexpressing WRKY6. This regulation is specific to the Trx h family.

It is becoming increasingly evident that ROS (H$_2$O$_2$) production and transcriptional regulation of redox-regulated genes are controlled by the circadian clock. These findings point to a possible functional link between ROS signalling and circadian output, which provides a mechanistic link for the plant response to oxidative stress. In fact, CCA1 (CIRCADIAN CLOCK-ASSOCIATED 1) seems to be a
central regulator for the co-ordinate relationship between the ROS-driven transcription, ROS production, and scavenging in response to oxidative stress (Lai et al., 2012). Among these genes, the circadian regulation of both chloroplastic \( f \) and \( m1 \) pea Trxs through control of the CCA1 TF was apparent, probably through its direct interaction with an evening element of these genes. This circadian regulation of Trxs can provide an additional regulatory mechanism that might be related to their redox regulation of light-dependent protein targets (Barajas-López et al., 2011). Transcript data reported that the abundance of \( AtTrx-like5 \) mRNA (WCRC Thioredoxin1) and \( AtTrx2f \) responded to oscillatory regulation (Harmer, 2009). Besides light, the expression of \( PsTrxsf \) and \( m1 \) is regulated by sugar, probably through a DOF 7 TF. However, the redox signalling pathways leading to changes in gene expression remain elusive (Barajas-López et al., 2012).

Many plant hormones and cellular redox agents are of great importance in the regulation of gene transcription under plant acclimation to stress. A number of excellent reviews have been published on the interplay between the signalling pathways (e.g. Astier et al., 2011; Bartoli et al., 2013). Thus, this interaction is not considered further here. As a representative example, osmotic stresses (drought, salinity) induce the expression of AZF1 and AZF2 (Arabidopsis zinc-finger proteins) via ABA-dependent or -independent pathways (Kodaira et al., 2011). When an amount of AZF1 and AZF2 TFs accumulates under stress, many ABA-repressive genes are suppressed. AZF1 and AZF2 interact with the promoter regions of several small auxin-up RNA (SAR) genes. Both TFs regulate ABA and auxin signalling in plant responses to osmotic stress. Recently, we described AZF2 as one of the putative TFs regulating \( AtTrxo1 \) gene expression (Ortiz-Espin et al., 2013). Further work may allow us to assess whether and how this redox-regulated TF operates in vivo to influence \( Trxo1 \) expression, which is also involved in redox regulation under plant development and/or the response to stress conditions (Martí et al., 2011).

The transcriprional regulation of the \( At2CysPrx \) \( A \) gene (2CPA) responds to ABA and chloroplast signals. This gene is induced by peroxides and photo-oxidative stress and repressed by ABA, and the regulation serves as a model to study redox and hormonal signalling pathways independently (Baier et al., 2004; Hilscher et al., 2014). Moreover, 2CPA expression is highly sensitive to ascorbate, is light-dependent, and seems to need the co-existence of the TFs Rap2.4 and RCD1 (Shaikhali et al., 2008; Shaikhali and Baier, 2010) (see below).

**ROS-responsive TFs**

Until the late 1980s, no evidence had been found that sulfenic acids, in addition to their role in catalysis and protection against ROS, can act directly as signalling molecules triggering the cell response to oxidative stress via TFs (Zheng et al., 2001) and allowing acclimation to specific stress conditions (Locato et al., 2010). The DNA-binding activity of numerous TFs in microorganisms and yeast, such as OxyR, OhrR, AP1, and CrfI, is regulated by the primary formation of a sulfenic acid, which is often transformed into a disulfide bond (Zheng et al., 1998; D’Autreux and Toledano, 2007) or modified by S-nitrosylation. Escherichia coli OxyR was shown to undergo different oxidative modifications in Cys199 such as -SOH, -SSG and -SNO, which differentially affected its DNA-binding capacity (Kim et al., 2002). Similarly, CrfI was modified on Cys420 after exposure to \( \text{H}_2\text{O}_2 \) or oxygen (Cheng et al., 2012). In mammals, the nuclear erythroid 2 related bZIP TF (Nrf2) in its inactive form is bound to the protein Keap1, the redox sensor of the complex that promotes its ubiquitination. \( \text{H}_2\text{O}_2 \) oxidizes reactive Cys of Keap1 and changes its conformation, breaking the complex and releasing Nfr2 in its active form. After phosphorylation, Nfr2 is translocated to the nucleus, where it binds to the antioxidant responsive elements of DNA and promotes the expression of antioxidant proteins such as Trx, TR, Prxs, glutaredoxins, and Srxs, which in turn eliminate the redox signalling (Flohé and Flohé, 2011). In yeast, the Prx Tpx1 has been shown to be necessary for the \( \text{H}_2\text{O}_2 \)-dependent activation of the bZIP TF Pap1 in a concentration-dependent manner (Schippers et al., 2012). This provoked its translocation to the nucleus and the activation of antioxidant genes. Moreover, the recycling of Tpx1 by the cytosolic-reduced Trx1 is required (Calvo et al., 2013). However, to date, few examples of such signalling functions are known in plants, although some redox-regulated TFs under retrograde regulation have been described under the stress response (Gupta and Luan, 2003).

As well as the above-described involvement of Trxh5 in the redox regulation of NPR1, allowing its translocation to the nucleus to activate redox-sensitive TGA TFs (Mou et al., 2003), sugarcane Trxh1 has been identified as an interacting partner of the redox-regulated TF SsNAC23. It is a member of the plant-specific NAC TF family, with roles in the development and response to cold stress (Jensen et al., 2010). The regulation seems to affect disulfide bridges between some of the three cysteine residues in the TF. Thus, Trxh1 might act by changing the structure of SsNAC23, with implications for its interactions with other proteins (Jensen et al., 2010). However, the exact molecular mechanism is not clearly described.

As another example, the redox-sensitive Rap2.4a, an AP2-type transcription factor, was identified as the first signal transduction component involved in redox regulation of the chloroplast 2CPA gene promoter mentioned above. Upon moderate redox imbalances, Rap2.4a protein dimerizes and binds to the promoter region activating 2CPA transcription. Under severe oxidative stress, this TF oligomerizes, loses its DNA affinity, and thus decreases transcription levels of the gene. Rap2.4 also orchestrates the activation of other genes encoding chloroplast antioxidant enzymes, including stromal ascorbate peroxidase (sAPX) and Cu,Zn-SOD (Shaikhali et al., 2008). The TF RCD1 (RADICAL-INDUCED CELL-DEATH 1) protein interacts with Rap2.4a and potentiates its effect in the transcriptional activation of genes encoding...
chloroplast antioxidant enzymes (Hiltscher et al., 2014). RCD1 also interacts with DREB2A TF in drought responses (Vainonen et al., 2012).

Redox control of eukaryote MYB TFs is through the two conserved Cys residues, which, under oxidizing conditions, form an intramolecular disulfide bond that alters its domain structure, preventing DNA binding. For clusters of MYB proteins in Arabidopsis presenting only one Cys residue, an alternative mechanism that involves cysteine S-nitrosylation may control and negatively influence their DNA-binding activity (Dubos et al., 2010). Other redox-regulated TFs in plants are heat-shock factors (Hsfs), which bind to the conserved heat-shock elements. Arabidopsis HsfA1a, directly senses heat stress and H\textsubscript{2}O\textsubscript{2} via redox state (Liu et al., 2013). This TF has two Cys residues that, when substituted for Ser, suppressed the expression of its target gene (Jung et al., 2013). The expression of HsfA2 and its target genes, APX2 and heat-shock protein 18.1-C1, is low under non-stressed conditions but is strongly induced by heat shock and ROS in Arabidopsis (Nishizawa et al., 2006; Shigeoka and Maruta, 2014). Another example is the TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1 (TCP), which participates in plant cell proliferation and growth. It contains a Cys sensitive to redox conditions and is inhibited under oxidative stress. The inhibition can be reversed by Trx and Trx reductase (Viola et al., 2013). An additional role of the modification of class I TCP proteins by redox agents may be their protection from permanent inactivation under oxidative stress conditions, considering that disulfide bond formation and modification by GSH, H\textsubscript{2}O\textsubscript{2}, or NO are reversible processes.

In conclusion, the above results suggest that redox changes operate in vivo to influence the activity of different TFs within plant cells. These redox-dependent modifications have an impact on downstream events regulated by each specific TF. This mechanism is thought to be one of the most important ways for the plants to respond and adapt to stress conditions.

**Future perspectives**

ROS and NO play a pivotal role in regulating numerous responses to biotic and abiotic stresses in plants. The involvement of the crosstalk between ROS and NO in the regulation of protein activity and function and the effect on signalling affecting gene transcription in response to different stresses is beginning to be elucidated. While there has been important progress in the signalling role of the redox state of thiol in Trxs and Prxs through PTMs such as protein oxidation, S-glutathionylation, and S-nitrosylation/denitrosylation, many challenges remain regarding thiol specificity. When and how they are co-ordinated to allow specific proteins to respond and their repercussions in the regulation of targets proteins and/or specific genes is a challenging task (Fig. 3). In addition, some Trxs could promote trans-nitrosylation and reduce S-nitrosothiols; thus, the characterization of plant Trxs as denitrosylases is important to establish their role in NO signal transduction. The introduction in plants of new Trx/Prx/Srx variants mimicking these PTMs in specific thiols may help to unravel the role of these proteins as redox sensors and in the signalling process under normal and stress conditions.

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**Fig. 3.** Under nitrosative or oxidative stress in the plant cell, Trx and Prx can be S-nitrosylated to protect them from overoxidation and to allow signalling. On the other hand, Prx can be overoxidized to the sulfenic form to function as a redox sensor. This form can be regenerated to the reduced one by the action of Srx and Trx. Prxs share a common catalytic mechanism where, by reducing peroxide, the catalytic active Cys is oxidized to a sulfenic acid (SOH), which then forms a disulfide bond with resolving Cys that is reduced by the Trx system. Under severe oxidative stress, Prxs overoxidize to the sulfenic form (SOOH), inactivating the peroxidase activity but gaining a chaperone activity. Ferredoxin/Trx system: Fd/FTR (ferredoxin/ferredoxin-dependent Trx reductase); NADPH/Trx system: NADPH/NTR (NADPH-dependent Trx reductase). (This figure is available in colour at JXB online.)
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