Review Article

Cysteine thiol-based post-translational modification: What do we know about transcription factors?

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GLOSSARY

Reactive oxygen species (ROS)

A class of highly reactive molecules derived formed from molecule oxygen by enzymatic and nonenzymatic reactions. ROS include including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot -}$), hydroxyl radical (HO•), singlet oxygen ($^1O_2$), and various forms of organic and inorganic peroxides.

Reactive nitrogen species (RNS)

A class of reactive molecules derived nitric oxide (NO), including nitrous oxide (N_2O), peroxynitrite ($ONOO^-$), and nitroxyl anion (NO^-), as well as other types of nitrogen-containing reactive free radicals.

Reactive sulfur species (RSS)

RSS were initially defined as a class of redox metabolites that can adapt the redox state of thiols and disulfides *in vivo* under oxidative stress conditions . However, many sulfur-containing molecules compound are synthesized under non-oxidative conditions and reduced forms of sulfur like hydrogen sulfide (H_2S) can react with protein thiols. Hence, RSS were redefined a class of redox-active, sulfur-containing molecules that are either oxidize or reduce biomolecules under physiological conditions. Thus far, as the prototypical inorganic RSS with a half-life on the minute time scale, H_2S is most studied in plants , and considered as an important signaling molecule participating in a wide range of physiological processes.

Post-translational modifications (PTMs)

PTMs are chemical alterations of amino acid side chains, such as the covalent addition of chemical groups such as acetyl, phosphoryl, glycosyl or methyl groups. PTMs are mostly enzymatically catalyzed, reversible or irreversible and can influence protein functions in various manners.

Cyseine thiol-based OxiPTMs

Thiol groups of cysteine (Cys) residues often reside in functional sites of proteins and are susceptible to oxidation by reactive, electrophilic species. Oxidative post-translational modifications (OxiPTMs) of Cys thiols such as *S*-sulfenylation, *S*-sulfinylation, *S*-glutathionylation, *S*-nitrosation, persulfidation acylation will result in different oxidation states of the Cys sulfur atom and are often collectively referred to as ‘Cys oxidation’. Note that Cys thiols also can

react with other biomolecules such as cyanide (HCN) or fatty acids, referred to as S-cyanylation or S-acylation. In this review, we mainly focus on H_2O_2/NO/H_2S-triggered OxiPTMs.

Abstract

Reactive electrophilic species are ubiquitous in plant cells, where they contribute to specific redox-regulated signaling events. Redox signaling is known to modulate gene expression during diverse biological processes, including plant growth, development, and environmental stress responses. Emerging data demonstrates that transcription factors (TFs) are a main target of cysteine thiol-based oxidative post-translational modifications (OxiPTMs), which can alter their transcriptional activity and thereby convey redox information to the nucleus. Here, we review the significant progress that has been made in characterizing cysteine thiol-based OxiPTMs, their biochemical properties, and their functional effects on plant TFs. We discuss the underlying mechanism of redox regulation and its contribution to various physiological processes as well as still outstanding challenges in redox regulation of plant gene expression.

Keywords: redox; plant; post-translational modifications; transcription factors

ROS/RNS/RSS in plants

Oxidation-reduction (redox) reactions are essential in cellular metabolism and are inevitably associated with the production of reactive electrophilic species, such as **reactive oxygen**, **nitrogen**, and **sulfur species** (**ROS/RNS/RSS, see Glossary**) [1-4]. In plants, ROS/RNS/RSS are specifically produced in different organelles through multiple pathways and their active contribution to redox signal transduction in diverse biological processes such as growth, development, stress responses and cell death is well documented [5–7]. Most ROS/RNS/RSS can diffuse across membranes and exert relatively high cross-reactivity with each other to generate other oxidants [8–10]. Reactions of these reactive electrophilic species are strongly dependent on their local concentrations and those of proximal antioxidant capacities (e.g. scavenging enzymes and metabolites), thus providing a dynamic and highly-specific regulatory framework for cellular signaling transduction. As such, these reactive molecules have long attracted attention in plant science.

Cys thiol-based OxiPTMs and feedback regulation

The regulatory functions of ROS/RNS/RSS are largely exerted by through oxidative post-translational modifications (OxiPTMs) of proteins [4,8,9,11]. In plants, H_2O_2, NO, and H_2S are most studied ROS/RNS/RSS. Within cells, they can react with particular Cys thiols to form *S*-sulfenylated, *S*-nitrosated, or persulfidated residues, respectively (see Figure 1 for an overview of OxiPTMs) [12–17]. These redox regulation affect the redox state of many functional proteins and fine tune plant development and stress response.

Interestingly, the enzymes responsible for the production of H_2O_2, NO, and H_2S are themselves target of OxiPTMs, acting as a feedback regulation to amplify or tone down their activity. For example, the NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) is subject to a negative feedback loop by *S*-nitrosation at a conserved Cys (Cys890), inhibiting ROS production during defense responses [18]. In contrast, persulfidation of RBOHD at Cys825 and Cys890 promotes ROS production during abscisic acid (ABA) dependent stomatal closure [19]. As another example, the activity of the major H2S biosynthesis enzyme, cytosolic L-CYSTEINE DESULFHYDRASE1 (DES1), is positively regulated by persulfidation of Cys44 and Cys205, thus providing a self-activation mode [19]. Conversely, NO-induced *S*-nitrosation of nitrate reductase 2 (NR2), the main enzymatic resource of endogenous plant NO production, potentially inhibit its activity [20]. Lastly, both *S*-sulfenlyation and *S*-nitrosation can inhibit the activity of S-NITROSOGLUTATHIONE

REDUCTASE 1 (GSNOR1), the enzyme regulating the turnover of the natural NO biosource **S-nitrosoglutathione** (**GSNO**), thereby further increasing NO content [21,22].

OxiPTMs fine-tuning the function of TFs in plants

Transcription factors (TFs) are a large and diverse protein family that regulate transcription by binding to specific DNA motifs in the promoter regions of target genes. TFs are vital for basal transcription supporting routine metabolism and development, as well as for launching transcriptional changes in response to various environmental stimuli.

H_2O_2/NO/H_2S are known to trigger genome-wide transcriptional changes upon abiotic and biotic stresses [23–26]. Concomitantly, *S*-sulfenylation, *S*-nitrosation, and persulfidation have been reported to directly affect the activity of TFs in plants [11,27]. OxiPTMs can influence distinct aspects of TF function, such as their subcellular localization, stability/structure, protein–protein interactions and DNA binding capacity, thereby resulting in gene expression reprogramming (Table 1).

Proteomic analyses identified a large number of plant proteins susceptible to cysteine thiol-based OxiPTMs [28–34]. A total of 56 TFs have at least one reported Cys OxiPTM site (e.g. -SOH, -SNO), as outlined in the Plant PTM Viewer resource (https://www.psb.ugent.be/webtools/ptm-viewer/) [35]. This most likely is an underestimation due to the very low protein abundance of TFs, which challenges their proteomic detection. Identification of thiol-based OxiPTMs can inspire downstream functional studies, which remains pivotal to study their roles on protein function and biological relevance. Here, we review such functional studies of plant TFs, discussing the redox regulation of cysteine thiol-based OxiPTMs on the TFs in plant immune responses, phytohormone signaling, development and abiotic stresses.

Plant immune responses

ROS and NO are produced upon pathogen perception by plasma membrane localized RBOHs and cytosolic NR, respectively [36,37]. Cysteine thiol-based oxiPTMs on several TFs have been shown to alter the expression of downstream defense genes, thereby fine-tuning plant immunity responses [38,39].

NPR1 and TGA1

Salicylic acid (SA) is an important defense hormone that balances ROS

production and scavenging [40]. The function of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1), a master regulator of SA-dependent plant immune responses, is affected by the cellular redox state (Figure 2) [41]. Under control conditions, NPR1 exists as a disulfide-based oligomer facilitated by both Cys82 and Cys216. Upon pathogen infection, intermolecular disulfide bonds are reduced and released NPR1 monomers that can shuttle to the nucleus [42]. In nucleus, monomeric NPR1 interacts with members of the clade I TGA sub-family of basic leucine zipper (bZIP) TFs and stimulates their DNA-binding activities [43]. This family of TFs recognizes the TGACG/*as-1*/*ocs* elements in the promoters of a variety of plant pathogen-associated and SA marker genes, including *PATHOGEN RELATED 1* (*PR-1*). However, a recent paper reported NPR1 has no disulfide bond regardless of SA treatment in planta, which challenges the NPR1 dogma [44].

Intriguingly, clade I TGA TFs sub-family members are redox-regulated as well. Under unstressed conditions, TGA1 contains an intramolecular disulfide bridge between Cys260 and Cys266 that structurally prevents its interaction with NPR1. However, SA-dependent redox changes facilitate the reduction of TGA1 by TRX upon pathogen infection, enabling its interaction with NPR1 and thereby stimulating TGA1 DNA binding activity [45]. A similar redox-dependent interaction with NPR1 is also observed with TGA4 [45,46].

ROS and RNS cross-talk determine defense gene expression and is mediated through OxiPTMs in the NPR1/TGA1 system during plant immune responses. Upon pathogen challenge, early-stage SA induction promotes TRX-h3/h5-mediated release of monomeric NPR1 resulting in nuclear TGA1-NPR1 transcriptional activity [47]. These redox alterations induce *S*-nitrosation and further oligomerization of NPR1 that tone-down defense responses (Figure 2) [48]. Simultaneously, the Cys260 and Cys266 of TGA1 can also be *S*-nitrosated or S-glutathionylated, both of which subsequently prevents its intramolecular disulfide bond formation and promote DNA binding. This enhancement could further increase in the presence of NPR1 [46]. However, the contradictory observation of GSNO-induced oligomerization and nuclear translocation of NPR1 indicates the complexity of ROS/RNS cross-talk of NPR1 during plant immune response [46,48].

SRG1

By profiling transcriptional changes in response to elevated NO accumulation in arabidopsis (*Arabidopsis thaliana*), a rapidly induced C2H2 zinc finger-containing TF designated SNO REGULATED GENE 1 (SRG1) was

identified as a positive regulator of plant immunity [49]. Either *S*-nitrosation or mutagenesis of Cys87 in SRG1 was shown to impair its DNA binding activity thus affect SRG1-mediated transcriptional repression. Given the conservation of Cys87, *S*-nitrosation mediated repression could be a common redox regulation mechanism for C2H2 zinc finger-containing TFs.

bHLH

Cucurbitacin C, a triterpenoid secondary metabolite, can be induced in some varieties of cucumber in response to stress and enhances the resistance to pathogens and insects. Expression of Cucurbitacin C biosynthetic genes is controlled by two basic helix-loop-helix (bHLH) TFs, Csa5G156220 and Csa5G157230 [50], which were shown to be persulfidated *in vitro* [51]. Persulfidation increased their DNA binding activities to the promoter of the CuC biosynthesis gene *Csa6G088690.* In line with the observation, exogenous H_2S fumigation significantly improves the resistance of cucumber leaves to the pathogen *Phytophthora melonis* [51].

Brassinosteroid signaling: BZR1 and BIN2

Brassinosteroids (BRs) are a class of polyhydroxylated steroidal phytohormones that play fundamental roles in plant growth, development, and stress adaptation [52]. BR is perceived through the plasma membrane-localized receptor kinase BRASSINOSTEROID INSENSITIVE1 (BRI1), which promotes its association with the co-receptor BRI1-ASSOCIATED KINASE1 (BAK1) and thereby induces a phosphorylation cascade that activates BRASSINAZOLE-RESISTANT1 (BZR1) and BRI1-EMS-SUPPSSOR1 (BES1), two homologous TFs regulating expression of downstream BR-responsive genes [53]. In the absence of BR, the GSK3-like kinase BRASSINOSTEROID-INSENSITIVE2 (BIN2) phosphorylates both BZR1 and BES1, which suppresses their transcription activity and thus downstream BR gene expression.

Cross-talk between BR and H_2O_2 is noted through an OxiPTM dependent activation of BZR1 (Figure 3) [54,55]. Oxidation of BZR1 Cys63 enhances the interaction with AUXIN RESPONSE FACTOR6 (ARF6) and PHYTOCHROME INTERACTING FACTOR4 (PIF4), key regulators in auxin and light-signaling pathways, ultimately resulting in the activation of growth-related genes. In addition, the thioredoxin TRX-*h5* interacts and reduces BZR1. As such, overexpression of TRX-*h5* diminishes BZR1 transcriptional activity and leading to reduced quiescent center cell division in arabidopsis [54]. BZR1 also

regulates stomatal movement, a process where H_2O_2 is a rate-limiting secondary messenger [55, 56]. Here, BZR1 oxidation stimulates the its interaction with the bZIP TF G-BOX BINDING FACTOR2 (GBF2) that induces the expression of β-AMYLASE1 (BAM1), leading to guard cell starch degradation and subsequent stomatal opening (Figure 3) [55]. Both cases indicate that OxiPTMs do not directly regulate the DNA binding activity of BZR1, but rather influence its interaction with other transcriptional factors. We hypothesize that a similar redox regulatory mechanism for the homologous TF BES1 is in place, as BES1 interacts *in vivo* with TRX-*h5* (Figure 4) [54].

BIN2, the upstream kinase of BZR1 and BES1, is also redox regulated. BIN2 is *S*-nitrosated at Cys162 which inhibits its activity under GSNO treatment [57]. Heat stress or exogenous H_2O_2 induced the thiol-based oligomerization of BIN2 and decreases its kinase activity, while increasing its sensitivity to the GSK3 inhibitor [58]. In contrast, increased $^1O_2$ levels activate BIN2 and its interaction with BES1 [59]. Currently, the OxiPTMs responsible for BIN2 redox regulation still await further investigation. Moreover, BIN2 was shown to participate in BR immune responses by associating with TGA1/TGA4 [60]. The phosphorylation of TGA4 by BIN2 facilitates its degradation and inhibits its redox-based interaction with NPR1, thus negatively regulating plant immune responses.

Abscisic acid signaling: ABI4, ABI5 and SnRK2.6

Seed germination and post-germination developmental processes are important for the reproduction of plants. These processes are are tightly controlled by the interplays among phytohormone abscisic acid (ABA) and H_2O_2/NO/H_2S, in a OxiPTM-dependent manner [61,62]. In general, seed germination is inhibited by ABA and H_2S but promoted by NO and H_2O_2 [63–65].

Building a bridge between ABA and H_2S signaling in seed germination, persulfidation was shown to regulate the function of the TF ABSCISIC ACID-INSENSITIVE 4 (ABI4), a key positive transcriptional regulator in ABA signaling [66]. The persulfidation at Cys250 of ABI4 positively affects its binding to its cognate motif in the promoter of *MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 18* (*MAPKKK18*), thereby instigating ABA-dependent MAPK cascades (Figure 4). Interestingly, persulfidation at Cys250 also enhances protein stability of ABI4, which in turn inhibits seed germination and seedling establishment [63].

The S-nitrosation of bZIP TF ABI5 at Cys153 (Figure 4) promotes its interaction with E3 ubiquitin ligases, leading to ubiquitin-mediated proteolysis

of ABI5 and attenuated inhibition of seed germination and seedling establishment [67]. In the canonical ABA signaling pathway, ABI5 is functionally regulated via phosphorylation catalyzed by SUCROSE NONFERMENTING1-RELATED PROTEIN KINASES (SnRK2s) [68,69]. Interestingly, ABA-induced NO and H_2S bursts trigger the *S*-nitrosation and persulfidation of SnRK2.6, respectively [57,70]. Persulfidation of SnRK2.6 promotes its kinase activity and interaction with the TF ABA RESPONSE ELEMENT-BINDING FACTOR 2 (ABF2) to positively regulate ABA signaling. In contrast, *S*-nitrosation inhibits the kinase activity of SnRK2.6, which may desensitize ABA signaling in order to coordinate plant growth and stress response (Figure 4) [57]. Furthermore, the activity of protein phosphatases ABI1 and ABI2, negative regulators of SnRK2, is also dependent on its redox status, which is controlled by thiol peroxidases such as GPX3 and type II peroxiredoxin [71,72]. In addition, BIN2 is found to phosphorylate and stabilize ABI5 during ABA-mediated seed germination [73]. The fact that S-nitrosation at Cys162 inhibited BIN2 activity during seed germination [57], further highlights the important function of *S*-nitrosation in the interaction between BR and ABA signaling.

Plant abiotic stress responses

Similar to immune responses, plants rapidly produce a burst of ROS/RNS in response to abiotic stresses under adverse environmental conditions [1,74]. Likewise, these bursts can govern specificity in transcriptional reprogramming by inducing OxiPTMs on TFs involved in responses to extreme temperatures, oxidative and osmotic stress (Table 1).

bZIPs

bZIP is a large TF family reported in stress and phytohormone signal transduction in plants [75] and a subclade, the G-group of bZIPs, was identified as a redox-sensitive subfamily. For instance, arabidopsis G-group bZIPs members bZIP16, bZIP68, and G-BOX BINDING FACTOR1 (GBF1) are influenced by chloroplast redox changes following exposure to high light [76]. Their DNA binding activity was reduced by H_2O_2 treatment, but enhanced by treatment with the reducing agent dithiothreitol. While bZIP16 can form a disulfide-based dimer, bZIP68 undergoes *in vivo* oxidation and oligomerization after H_2O_2 treatment, which drives nucleus-to-cytosol translocation [77]. As the bZIP domain shares high similarity to other bZIP TFs, these results imply that similar redox regulation might hold true for other bZIP TFs.

Coincidently, redox regulation of a VRE-like bZIP TF in response to osmotic

stress has been reported in rice [78]. *Os*bZIP68 undergoes oxidation and homo-tetramerization mediated via Cys245 after treatment with H_2O_2 or polyethylene glycol, resulting in increased DNA binding to its downstream target genes. Moreover, the thiol peroxidase *Os*GPX1 was shown to enzymatically oxidize *Os*bZIP68 [78]. During osmotic stress, GPX1 forms an intramolecular disulfide bond at Cys71 and Cys90, gets transferred to induce *Os*bZIP68 Cys245-based oligomerization. In this case, GPX1 acts as a redox sensor to mediate the redox status change of bZIP68 in response to osmotic stress, pointing out the importance of enzyme-catalyzed OxiPTMs in redox signaling transduction (Figure 5).

DREB1/CBFs

To cope with low temperatures, plants have evolved dedicated and effective mechanisms to enhance their cold stress tolerance in a timely manner, known as cold acclimation. In this process, DEHYDRATION-RESPONSE ELEMENT-BINDING PROTEIN1/C-REPEAT BINDING FACTORS (DREB1/CBFs) have a critical role [79]. DREB1/CBF TFs recognize the DEHYDRATION-RESPONSIVE ELEMENTs (DREs) that are present in the promoters of cold-regulated genes. A recent study highlighted the significance of redox regulation in plant freezing tolerance [80]. Under low temperatures, TRX-*h2* was shown to re-localize from the cytoplasmic endomembrane to the nucleus where it reduces all oxidized oligomeric and monomeric CBF. This process renders all CBFs functionally active, and promotes cold-regulated gene expression to confer freezing tolerance in plants [80].

NPR1-HsfA1 and HsfA8

Heat stress TFs (Hsfs) regulate the expression of stress-responsive genes in multiple abiotic stress conditions [81]. In addition to the DREB1/CBFs, other transcriptional complexes, such as HsfA1/NPR1, are also regulated by OxiPTMs under cold stress [82]. Released monomers from NPR1 oligomers by the action of TRXH3 and TRXH5 were translocated into nucleus and interacted with different TFs including HsfA1 in response to cold stress. This NPR1-HsfA1 transcriptional complex activates HsfA1 target genes, including chaperones, that promote plant freezing tolerance [82]. In addition, HsfA8 is another redox-sensitive TF, with H_2O_2 treatment inducing the nuclear translocation of HsfA8 in Cys24 and Cys269 dependent manner [83].

GT-1

GT factors are TFs that promote the expression of light regulated genes in plant stress responses and development [84]. Recently, it was shown that NO bursts under heat stress led to *S*-nitrosation at Cys324 or Cys347 of GT-1, which is responsible for the NO-induced expression of *HsfA2*, thereby activating heat-responsive gene expression and thermotolerance [85].

HFR1

Light and high temperature have opposite effects on seed germination, while light induces, heat suppresses seed germination. Recently, *S*-nitrosation of LONG HYPOCOTYL IN FAR-RED1 (HFR1) offered a mechanistic link between light- and heat-controlled seed germination [86]. Light stabilizes HFR1 that forms a heterodimeric complex with PIF1 and inhibits its DNA binding activity, thereby promoting seed germination under light. However, high temperature induces *S*-nitrosation of HFR1 Cys164, resulting in its degradation and release of PIF1 that activates expression of the CCCH-type zinc finger protein SOMNUS which can alter GA and ABA metabolism and finally suppress seed germination [86].

Plant development

The production of reactive species is not restricted to environmental stress responses. Instead, they play essential roles in physiological processes driving plant growth and development. Flowering is a crucial developmental event in plants and is part of the transition from vegetative to reproductive growth. The vegetative shoot apical meristem (SAM) generates leaf whorls, branches and stems, whereas the reproductive SAM, or inflorescence meristem, forms florets arranged on a stem or an axis. These processes are tightly controlled by a series of TFs that are redox regulated by Cys thiol-based OxiPTMs (Table 1).

TMF

In tomato, *TERMINATING FLOWER* (*TMF)* encodes a TF that is functionally important in flowering regulation [87]. When *TMF* is mutated, the meristem maturation program is prematurely terminated due to a precocious activation of the F-box gene *ANANTHA* (*AN*), leading to early flowering and single-flower primary inflorescences. TMF and flowering transition was recently reported to be redox regulated in tomato [88]. Developmentally produced H_2O_2 in SAM triggers inter- and intramolecular disulfide bonds targeting four Cys of TMFs to varying degrees, thereby elevating the amount of intrinsically disordered regions (IDRs) to drive phase separation. The resulting TMF condensates bind

with promoter of *AN* and repress its expression. Accordingly, mutation of the four TMF Cys residues and IDR domain comprised the promoter binding ability of TMF to *AN* [88]. Such transcriptional condensates facilitated by disulfide bond formation poses an interesting redox regulatory mechanism that possibly is central to many other TFs in physiological processes.

FEA4

Redox regulation of a TGA TF influences IM development in maize. Specifically, deficiency of FASCIATED EAR4 (FEA4) in maize, led to a profound increase in IM size [89]. The conserved Cys321 of FEA4 is responsible for oxidation-induced dimerization. During ear development, FEA4 interacts with MALE STERILE CONVERTED ANTHER1 (MSCA1), a maize CC-type GRX, as well as its two paralogues, *Zm*GRX2 and *Zm*GRX5. The CCMC motif of MSCA1 and the conserved Cys321 of FEA4 are critical for their interaction. The redox status of FEA4 was tightly controlled by GRXs, as Cys321 of FEA4 is shown to be reduced by MSCA1. Both FEA4 dimerization and *GRXs* mutation enhances the DNA binding and transcriptional activity of FEA4 to auxin-related genes, thus leading to stronger suppression of IM activity [89].

FLC

The MADS box TF FLOWERING LOCUS C (FLC) is a major repressor of flowering genes such as *FLOWERING LOCUS T* (*FT*)*, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*)*, and FLOWERING LOCUS D* (*FD*) [90]. There are four FLCs in heading Chinese cabbage, among which *Bra*FLC1 and *Bra*FLC3 undergo persulfidation resulting in decreased binding to downstream genes *BraSOC1* and *BraFT*, respectively [91]. As a consequence, the expression levels of *BraFT* and *BraSOC1* homologs (*BraSOC1-2/3/5* and *BraFT2*) were upregulated by treatment with the H_2S donor sodium hydrosulfide, while a H_2S synthesis inhibitor (hydroxylamine) had the opposite effect [91].

TCP

Class I of TEOSINTE BRANCHED1-CYCLOIDEA-PROLIFERATING CELL FACTOR1 (TCP) TFs plays important roles in BR and auxin mediated cell proliferation and growth [92]. Their DNA binding activity is regulated by their redox status. Under oxidizing conditions, class I TCPs form dimers with decreased DNA binding ability, while dithiothreitol, GSH or TRX treatments re-store their monomeric status and DNA binding capacity [93]. Cys20 is the

critical site for the dimerization and redox sensitivity of TCP15 [93], and mostly conserved within class I TCPs. Furthermore, this OxiPTM-mediated inhibition was correlated with changes in the expression of its downstream gene *CUP-SHAPED COTYLEDON1*, which influences ovule numbers in arabidopsis [94].

VND7

VASCULAR-RELATED NAC-DOMAIN (VND) TFs are important transcriptional regulators of xylem vessel differentiation, a process which is regulated by *S*-nitrosation in arabidopsis [95]. Overexpression of VND7 induces xylem vessel cell differentiation, but this phenotype is suppressed by a mutation in *GSNOR1*. VND7 was shown to be *S*-nitrosated close to its transactivation domains at Cys264 and Cys320. GSNO application and mutagenesis of both Cys264 and Cys320 to tryptophan, mimicking *S*-nitrosation, decreased VND7 activity, indicating *S*-nitrosation suppresses VND protein function. However, replacing Cys264 and Cys320 by serine of VND7 also decreased its transactivation activity, suggesting these Cys are important in VND7 function.

Concluding remarks and future perspectives

Recent studies highlighted the complexity of redox signaling in regulating plant stress responses and developmental processes. During the early stages of a signaling cascade, redox signaling mediated by cysteine thiol-based OxiPTMs can immediately steer TF function. This is evident in different phytohormone pathways (*e.g.* SA, BR, and ABA signaling) and underscores the cross-talk between redox and phytohormone signaling (Figure 2-4). In BR and ABA signaling, both TFs as well as their upstream kinases are affected by OxiPTMs, exemplary of the multi-layered redox regulation and crosstalk among different PTMs in physiological processes. OxiPTMs can fine-tune TF function in several ways. It can determine the nucleocytoplasmic localization of TFs or their redoxins (*e.g.* TRX-*h2*), their interactions with other TFs, protein stability, or possibly drive phase separation to form transcriptional condensates thus modulating transcriptional efficiency (Figure 5). However, still many questions remain unanswered regarding the coordination between different TFs and their roles during plant physiological processes (see Outstanding questions).

OxiPTMs mainly result from non-enzymatic reactions, depending on local concentrations of reactive species, but recent evidence also strongly indicates the importance of enzyme-catalyzed OxiPTMs. As already mentioned, rice GPX1 was reported to sense H_2O_2 and oxidized the TF *Os*bZIP68 [78]. In addition, peroxiredoxin and quiescin sulfhydryl oxidase homolog (QSOX1)

serve as redox sensors and transducers that regulate plant stress response [72,96]. Moreover, transnitrosation/transpersulfidation events were discovered recently [17,22], raising the possibility that some proteins might also be regulated in an enzyme-dependent manner via "transferase" reactions. Thus, the discovery and characterization of the protein(s) exerting such functions will contribute to an in-depth understanding of plant redox signaling.

Cellular redox status is highly dynamic, and, TRXs/GRXs play critical roles in the reduction of oxidized TFs. However, TFs are rarely reported as TRX/GRX substrates via proteomic studies, highlighting the need for dedicated set-ups focusing on nuclear redox proteomes. Ultimately, fundamental research on cysteine thiol-based OxiPTMs controlling TFs can motivate the genome-editing of crops to make them more resistant to environmental stresses.

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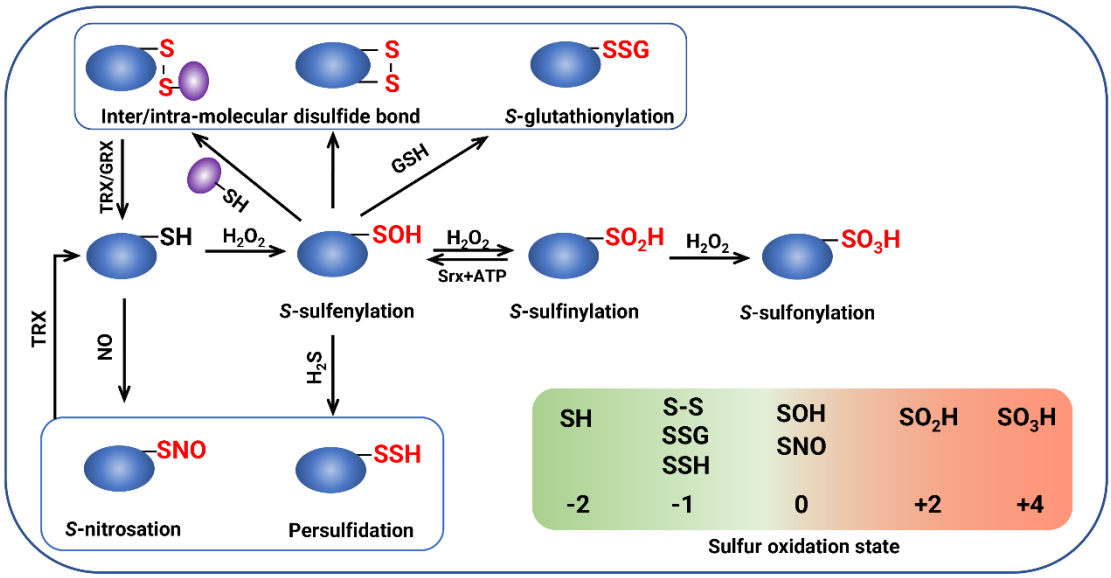

Figure 1. Schematic representation of biochemical interconversion of different OxiPTMs based on current researches. Protein Cys thiols (–SH) are intrinsically reactive and susceptible to oxidation by H_2O_2, the most stable amongst the ROS. The reaction between thiols and H_2O_2 initially forms sulfenic acid (R–SOH; *S*-sulfenylation), a transient OxiPTM that can be either reversed or serve as gateway towards other OxiPTM types. For example, –SOH can react with proximal –SHs within the same protein or from other proteins and glutathione (GSH), forming disulfides (–SSR) and S-glutathione (–SSG; *S*-glutathionylation), respectively. These disulfides can be enzymatically reduced by thioredoxins (TRXs) or glutaredoxins (GRXs) [12]. Alternatively, sulfenic acid (–SOH) can form sulfinic (–SO_2H; *S*-sulfinylation) and sulfonic (–SO_3H; *S*-sulfonylation) acids upon excessive H_2O_2 exposure. While, –SO_3H formation is irreversible and typically associated with permanent loss of function and protein degradation [13]. Specifically, it was found that –SO_2H on peroxiredoxins can be reduced by sulfiredoxin in the presence of ATP to –SOH [14,15]. Next to ROS triggered PTMs, *S*–nitrosation can be induced by NO, or other RNS such as $ONOO^-$, and results in the covalent addition of a NO group to reactive Cys thiols (–SNO). *S*-nitrosation can be reduced by TRXs, *i.e.* denitrosylation, or NO moieties can be transferred to other protein thiols - a process referred to as transnitrosylation [9]. Lastly, H_2S can reacts with oxidized thiol derivatives (*e.g.* disulfides or –SOH) to form persulfides (–SSHs), a process called persulfidation (or *S*-sulfhydration) [10,16], which can be further reduced by TRXs. Recently, transpersulfidation is also proposed as a plausible mechanism of persulfidation formation [17]. The respective sulfur oxidation states are indicated by a color code.

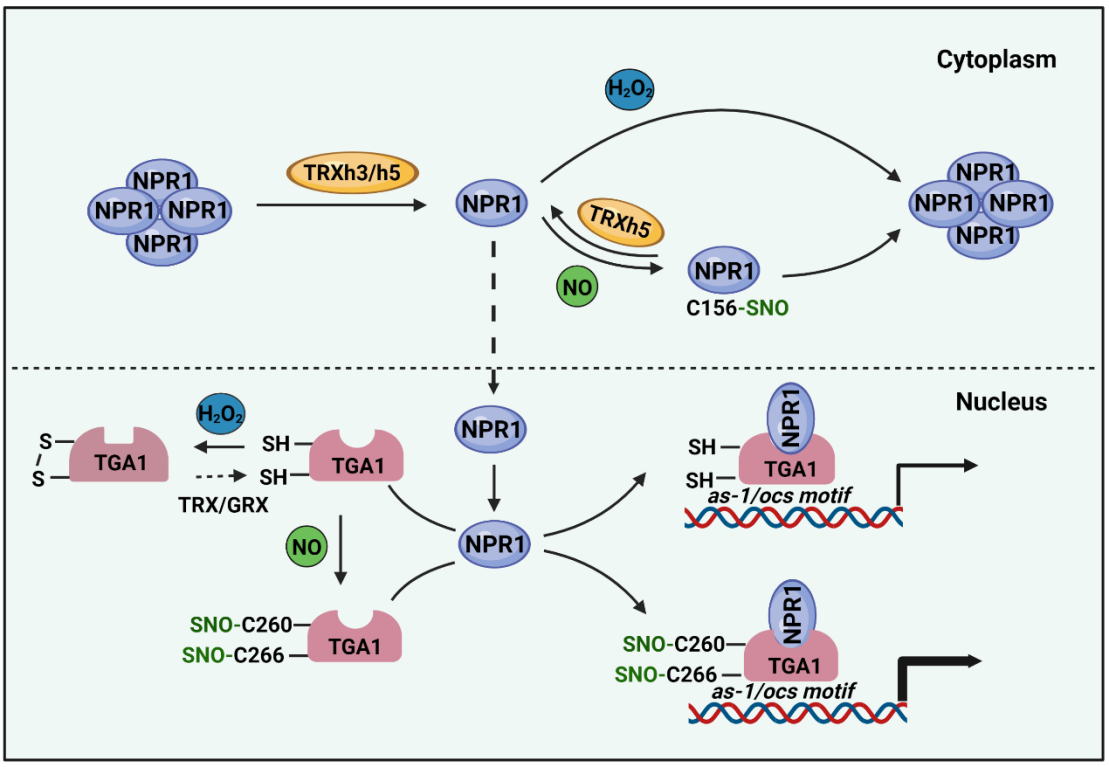

Figure 2. OxiPTMs regulations of NPR1 and TGA1 in plant immune response. In the absence of pathogen challenge, NPR1 exists as an oligomer in the cytoplasm with intermolecular disulfide bonds between monomers. In contrast, TGA1 is retained in the nucleus, with intramolecular disulfide bonds preventing binding to its target motif. Upon pathogen attack, a cellular redox change induces TRX-h3/5 mediated reduction of NPR1 oligomers to monomers, leading to NPR1 translocation from the cytosol to the nucleus. In addition, the redox change also reduces intramolecular TGA1 disulfide bonds, enabling physical interaction between NPR1 and TGA1, thereby activating downstream defense gene expression. Afterwards, ROS/RNS accumulation leads to the *S*-nitrosation and oxidation of NPR1, promoting its oligomerization form in the cytoplasm, while TRX-h5 can directly denitrosylate *S*-nitrosated NPR1 and promote its nuclear translocation. Furthermore, *S*-nitrosation of TGA1 prevents its disulfide bond formation and increasing its DNA binding activity in presence of NPR1.

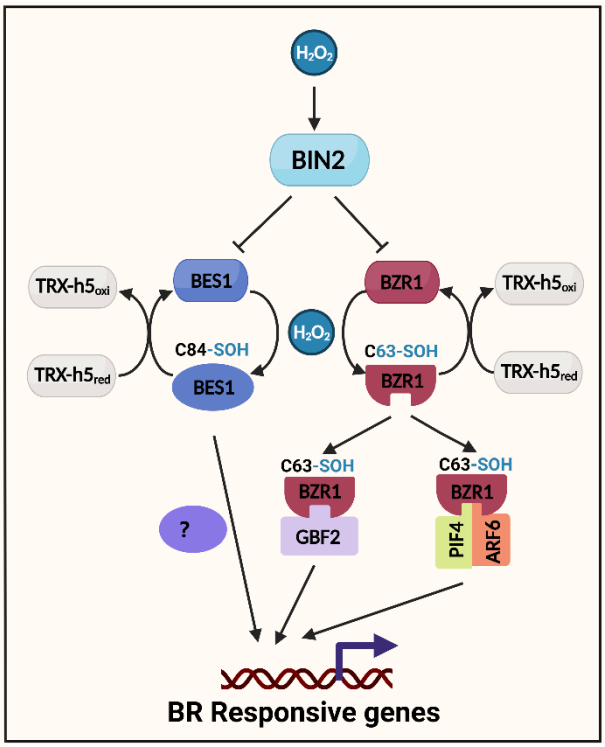

Figure 3. OxiPTMs in TFs of BR signaling pathways. H_2O_2-mediated oxidation activates BIN2 and enable it to associate with and phosphorylate BES1. As the key TFs in BR signaling, BZR1 is function regulated by oxidation. Oxidation in BZR1 enhance its interaction with GBF2, PIF4 and ARF6, thus activating downstream BR responsive gene expression. Whille the redox status of BES1 and BZR1 are controlled by TRX-h5, it remains unclear what and how TFs involved in oxidation-regulated BES1 function.

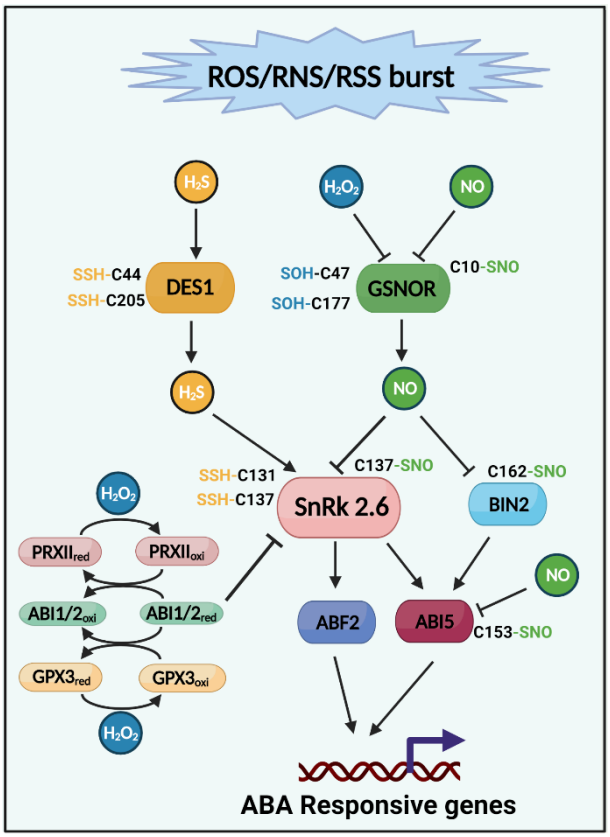

Figure 4. OxiPTMs in TFs of ABA signaling pathways. Reactive species accumulation is triggered when plant perceive environmental stimuli. These processes are always amplified by OxiPTMs. For example, accumulated H_2S could induce persulfidation of DES1, thus further promoting cytosol H_2S production. The burst of H_2O_2 and NO inhibited GSNOR activity via oxidation and *S*-nitrosation, thus increasing NO content. During ABA signaling, the activated SnRK2.6 phosphorylate and positively control the function of AREB/ABF TFs and regulate ABA responsive gene expression. SnRK2.6 activity is positively or negatively regulated by persulfidation or *S*-nitrosation, respectively. Persulfidation also enhances the ability of SnRK2.6 to interact with ABF2. ABI1/2-mediated phosphorylation are responsible for the inhibition of SnRK2.6 while ABI1/2 activity are regulated by its redox status, in which oxidation inhibits it phosphatase activity. GPX3 and PRXIIB may mediate the redox status of ABI1/2, thus mediating ABA signaling. BIN2 activity is also inhibited by *S*-nitrosation (Miao et al., 2006; Bi et al., 2022). Both SnRK2.6 and BIN2 are involved in the phosphorylation and activation of ABI5, while *S*-nitrosation facilitates the degradation of ABI5.

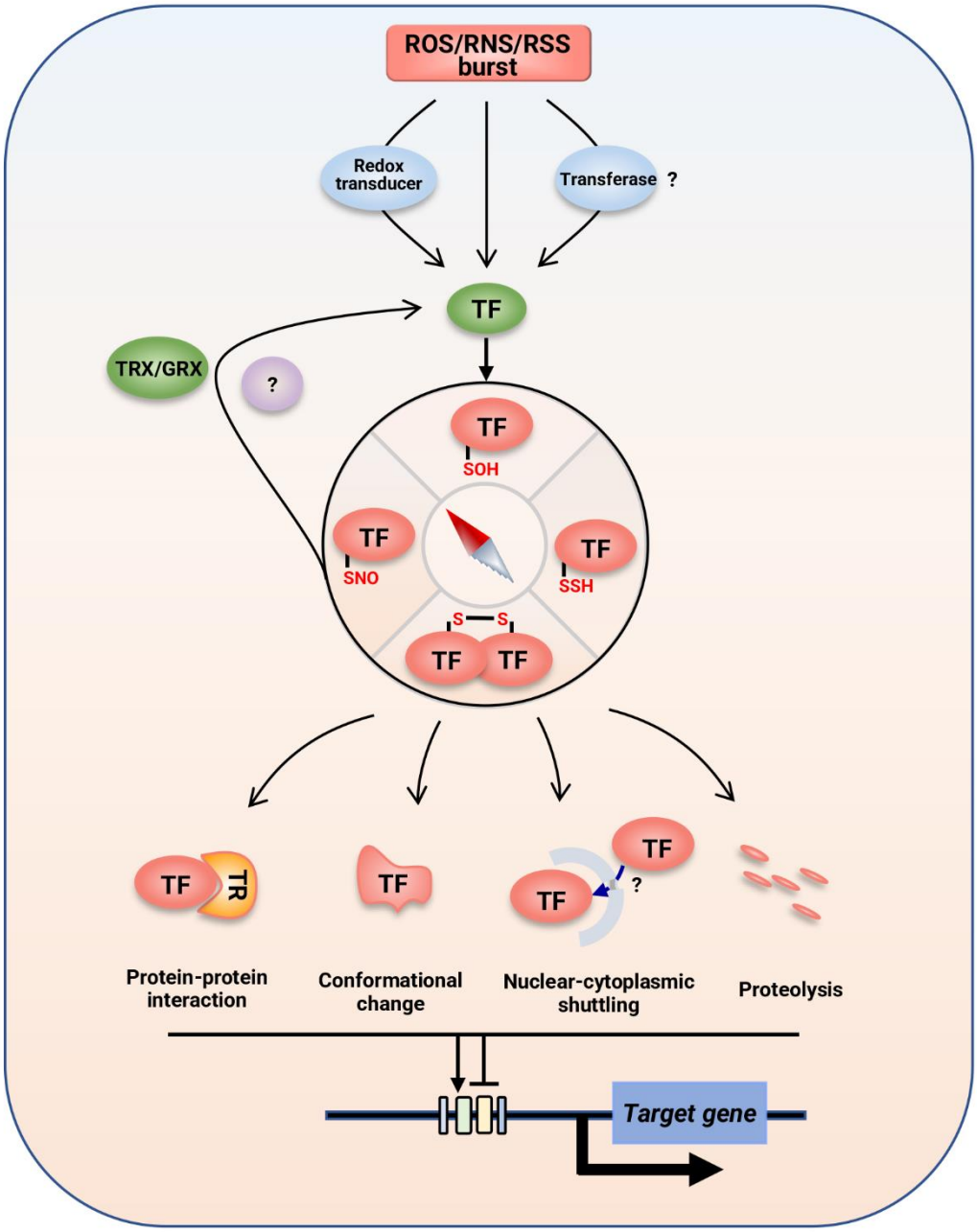

Figure 5. Schematic model of thiol-based OxiPTMs effect on TFs in plants. During plant stress and developmental response, the burst of ROS/RNS/RSS can trigger the oxidation of Cys thiols by direct react with TF or in an enzymatic-dependent way, such as redox transducer and transferase. Thiol-based OxiPTMs, including S-nitrosation, persulfidation, S-sulfenylation, and subsequent intra/intermolecular disulfide bond formation, regulate TF function mainly through four different pathways. First, redox PTMs affects the interactions between TF and corresponding transcriptional regulator, including

repressor and activator. Second, redox PTMs causes TF conformational change, including form inter- and intramolecular disulfide bond and drive phase separation to form transcriptional condensates, thus affecting its binding activity with promoter of target genes. Third, Redox PTMs enables TF nuclear localization which promote its role as TF by direct or interact with other binding partner to regulate target gene expression. However, it is still not clear for its underlying mechanism. Fourth, redox PTMs controls the turnover of TF by regulating its interaction with ubiquitin-proteasome system and thus affects transcriptional regulation.