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Review

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Regulating the regulator: nitric oxide control of post-translational modifications

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Summary

Nitric oxide (NO) is perfectly suited for the role of a redox signalling molecule. A key route for NO bioactivity occurs via protein *S*-nitrosation, and involves the addition of a NO moiety to a protein cysteine (Cys) thiol (–SH) to form an *S*-nitrosothiol (SNO). This process is thought to underpin a myriad of cellular processes in plants that are linked to development, environmental responses and immune function. Here we collate emerging evidence showing that NO bioactivity regulates a growing number of diverse post-translational modifications including SUMOylation, phosphorylation, persulfidation and acetylation. We provide examples of how NO orchestrates these processes to mediate plant adaptation to a variety of cellular cues.

I. Introduction

More than 200 reversible protein post-translational modifications (PTMs) have been identified to date, massively expanding the proteome and, by extension, enabling a plethora of protein functions (Minguez *et al.*, 2012), providing an escape from genetic incarceration. Typically, PTMs target amino acid residues embedded within conserved motifs (Tompa *et al.*, 2014). In this context, redox signalling is rapidly emerging as a key regulator of plant protein function associated with a myriad of plant processes. The small gaseous molecule, nitric oxide (NO), is a central player in redox signal transmission, mediating its redox functions predominantly through *S*-nitrosation/*S*-nitrosylation: the addition of a NO moiety to a cysteine (Cys) sulfhydryl/thiol to form an *S*-nitrosothiol (SNO) (Lindermayr *et al.*, 2005; Besson-Bard *et al.*, 2008b; Leterrier *et al.*, 2011; Yun *et al.*, 2016). This redox-based modification has been shown to regulate development, environmental responses and plant immunity. The emerging evidence suggests that NO orchestrates some of these processes through regulating the deployment of diverse PTMs. Here, we highlight some of these recent developments.

II. SUMOylation

SUMOylation, the covalent attachment of the small ubiquitin-like modifier (SUMO) to target proteins is emerging as a key modulator of eukaryotic immune function. In plants, SUMO1/2-dependent processes have been proposed to control the deployment of host immunity (Lee *et al.*, 2008a; van den Burg *et al.*, 2010; Saleh *et al.*, 2015). Recently, a key role for *S*-nitrosation in the control of SUMOylation has emerged (Skelly *et al.*, 2019). Following the pathogen triggered nitrosative burst, increasing NO levels were shown to drive *S*-nitrosation of Arabidopsis SUMO E2 enzyme, SCE1, at Cys139. The SUMO-conjugating activities of both SCE1 and its human homologue, UBC9, were both blunted by this PTM (Fig. 1a). Accordingly, mutation of Cys139 resulted in the accumulation of SUMO1/2 conjugates (Fig. 1b), disabled immune responses and increased pathogen susceptibility (Skelly *et al.*, 2019). Collectively, these findings established that *S*-nitrosation of SCE1 at Cys139 enables NO bioactivity to promote immune activation by relieving SUMO1/2-mediated suppression. This discovery is important because it suggests a new paradigm for the regulation of SUMOylation. The global control of this PTM is predominantly thought to occur at the level of each substrate via complex local machineries (Bossis & Melchior, 2006). By contrast, these new findings uncovered a novel, parallel and complementary mechanism by establishing that total SUMO conjugation is additionally regulated directly by SNO formation at SCE1 Cys139. Significantly, this Cys residue is evolutionary conserved and specifically *S*-nitrosated in human UBC9, implying that this immune-related regulatory process might be conserved across phylogenetic kingdoms (Skelly *et al.*, 2019). Therefore, NO bioactivity conveyed through *S*-nitrosation is a key regulator of SUMOylation, a ubiquitous eukaryotic PTM.

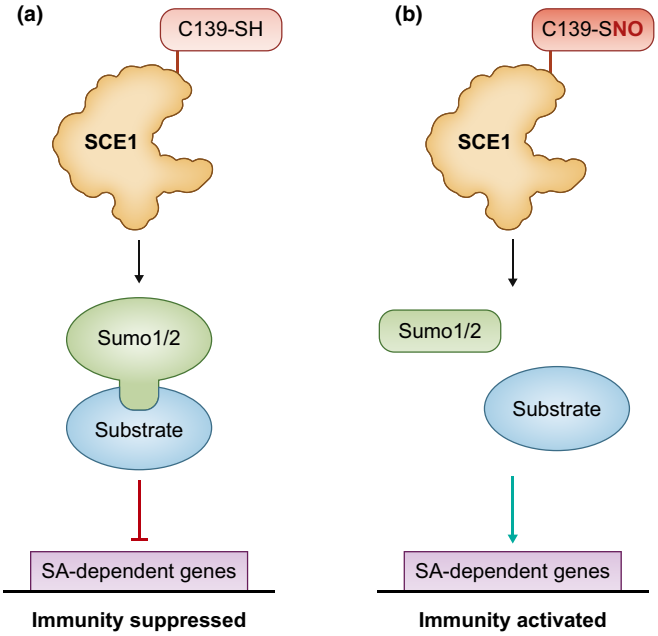

Fig. 1 Nitric oxide (NO) regulates SUMOylation through *S*-nitrosation of SUMO-conjugating enzyme. (a) In the absence of NO, SUMO (small ubiquitin-like modifier) conjugating enzyme (SCE1) SUMOylates key substrates with SUMO1/2 contributing to the repression of salicylic acid (SA)-dependent genes and, by extension, the suppression of immunity in the absence of pathogens. (b) Pathogen recognition triggers a nitrosative burst leading to NO accumulation, which results in the *S*-nitrosation of SCE1 at cysteine (Cys)139. This redox-based post-translational modification inhibits SCE1 activity blocking SUMO 1/2 SUMOylation. Consequently, this enables the expression of SA-dependent genes and the subsequent activation of plant immunity.

III. Phosphorylation

The emerging data suggest that NO is also a major regulator of phosphorylation-dependent signalling cascades. NO accumulation can trigger the activation of protein kinases (PKs) as well as the phosphorylation of numerous proteins related to diverse cellular processes (Besson-Bard *et al.*, 2008a; Frederickson Matika & Loake, 2014; Del Castello *et al.*, 2019). NO-dependent PKs include Ca^{2+}-dependent PKs (CDPKs), sucrose nonfermenting 1-related PKs (SnRKs), mitogen-activated PKs (MAPKs) and phosphoinositide-dependent PKs (PDKs). However, the mechanism(s) by which NO modulates the activity of these target PKs remain unclear.

NO is thought to indirectly mediate the activation of MAPKs and CDPKs through the mobilisation of cytosolic free Ca^{2+} (Besson-Bard *et al.*, 2008b). Yet, the subtle mechanisms underlying this process also remain to be determined. Direct *S*-nitrosation has not been confirmed for SnRKs (Wawer *et al.*, 2010), nor reported for MAPKs or CDPKs. However, the activity of tomato cell-death regulator PDK1 was found to be inhibited by *S*-nitrosation of a critical catalytic Cys residue. Additionally, the activity of MAPKs may be modulated by tyrosine nitration, as suggested by preliminary experiments (Ling *et al.*, 2012). Indeed, MAPKs become activated by MAPK kinases (MAPKKs) through dual phosphorylation of the Thr–X–Tyr motif in the activation loop. It is therefore

tempting to speculate that nitration of the Tyr residue within the activation loop could interfere with its phosphorylation by MAPKKs and, consequently, negatively modulate MAPK activity.

Finally, NO might modulate phosphorylated PK and, more generally, phosphorylated proteins through the redox regulation of protein phosphatases (PPs). This process is well established in animals and affects major phosphatases, including tyrosine phosphatases (Nakamura & Lipton, 2019). In this context, either activation, inhibition or a protective effect of the PP against oxidation-induced inactivation has been observed, depending on the specific PP. However, to date, no NO-dependent PP has been characterised in plants. So, this would be an interesting area for future exploration.

More generally, it is tempting to speculate that the post-translational modification of residues by NO or NO-derived compounds could trigger steric hindrance, altering the interaction with and phosphorylation by upstream kinases. For instance, *S*-nitrosation of the phosphotransfer protein AHP1, involved in cytokinin signalling, suppresses its phosphorylation, repressing cytokinin signalling (Feng *et al.*, 2013). The reciprocity of this mechanism could also be possible: phosphorylation of a given protein could also impact its subsequent *S*-nitrosation.

IV. Histone acetylation and methylation

Chromatin structure in eukaryotic organisms is very dynamic and is altered during growth and development and in response to environmentally stimuli. Modification of histone proteins induces chromatin remodelling to control transcription, replication, recombination and repair (Bannister & Kouzarides, 2011). Adjustment of histone acetylation or methylation, catalysed by histone acetyltransferases/histone deacetylases (HDAs) and methyltransferases/demethylases, respectively, are integral to these processes (Servet *et al.*, 2010; Shen *et al.*, 2015). Recently, it has been demonstrated that NO affects histone acetylation by targeting and inhibiting histone deacetylase (HDA) complexes (Mengel *et al.*, 2017). Genome-wide NO-dependent H3K9/14ac profiling in Arabidopsis seedlings identified NO-regulated histone acetylation of genes integral to immunity, abiotic stress and chloroplast function, suggesting that NO bioactivity might regulate gene expression by modulation of chromatin structure (Mengel *et al.*, 2017). A direct effect of NO on enzymes catalyzing DNA or histone methylation/de-methylation in plants has not been reported. However, genes encoding these enzymes are induced by NO or differentially expressed in plants with impaired NO homeostasis (Shi *et al.*, 2014; Hussain *et al.*, 2016; Kovacs *et al.*, 2016). Moreover, NO accumulation has been shown to induce global DNA hypomethylation, resulting in altered expression of chromatin remodelling enzymes (Ou *et al.*, 2015). This implies an indirect effect of NO on chromatin methylation mechanisms in plants. Overall, the emerging data suggest that NO bioactivity might play important roles in the nucleus, however, the molecular details still require further investigation.

V. Crosstalk between NO, ROS and H_2S

Nitro-fatty acids are reactive signalling mediators that are formed when unsaturated fatty acids, typically oleic or olenic acid, react with NO or reactive nitrogen species (RNS) (Kelley *et al.*, 2008; Corpas *et al.*, 2013). Recently, nitro-oleic acid has been found to activate NADPH oxidase (RBOH), altering reactive oxygen species (ROS) production (Arruebarrena *et al.*, 2020); this implies a novel signal link between NO-based and ROS-based signalling. It is already well established that the isoenzyme RBOHD is *S*-nitrosated at Cys890 inhibiting the activity of this enzyme and thus curbing pathogen-triggered oxidative burst to limit the extent of HR-associated cell death (Yun *et al.*, 2011). Additionally, the main enzymatic source of peroxisomal hydrogen peroxide (H_2O_2), glycolate oxidase, is also inactivated by *S*-nitrosation (Ortega-Galisteo *et al.*, 2012) and possibly also nitration (Lozano-Juste *et al.*, 2011), suggesting dual NO-dependent regulation. NO-based PTMs may also affect several ROS scavenging enzymes and some of these, for example, ascorbate peroxidase (APX) and superoxide dismutase (SOD), were found to be inversely regulated by *S*-nitrosation and nitration (Yang *et al.*, 2015; Kolbert & Feigl, 2017). Thus, NO-related PTMs may act as an on–off switch for antioxidant enzyme activities.

In addition to NO, hydrogen sulphide (H_2S) and H_2O_2 are also recognised as redox signal molecules in both animal and plant cells. They can also affect protein function through their redox interactions with critical thiols (–SH) on side groups of Cys residues, leading to PTMs. H_2O_2 causes oxidation of cysteinyl thiols to sulfenic acid, also identified as S-sulfenylation (Huang *et al.*, 2019), whilst H_2S results in persufidation (Hancock, 2019; Corpas *et al.*, 2019). Surprisingly, many of the targets for these molecules are key enzymes involved in ROS metabolism (Table 1).

In summary, the emerging evidence suggests that NO-related PTMs modulate enzymes involved in both ROS production and scavenging, suggesting that NO tightly regulates ROS homeostasis. Beyond direct protein modifications, NO may also compete for direct targets of both ROS and H_2S-based PTMs, indicating the possibility of multilevel regulation.

Table 1 Representative examples of enzyme involved in reactive oxygen species (ROS) metabolism whose activities are regulated by both nitric oxide (NO) and hydrogen sulfide (H_2S).

Enzyme	NO	H_2S	References
Ascorbate peroxidase (APX)	Activity upregulated	Activity upregulated	Begara-Morales *et al.* (2014); Aroca *et al.* (2015)
Catalase	Activity downregulated	Activity downregulated	Ortega-Galisteo *et al.* (2012); Corpas *et al.* (2019)
Respiratory burst oxidase homologue protein D (RBOHD)	Activity downregulated	Activity upregulated	Yun *et al.* (2011); Shen *et al.* (2020)

VI. NO regulation of the N-end rule protein degradation pathway

Transcriptional responses to reduced oxygen (hypoxia) are achieved by oxygen-dependent degradation by the ubiquitin proteasome system (UPS) of transcription factors mediated through the N-end rule (Gibbs *et al.*, 2016; Dissmeyer *et al.*, 2018). This pathway of targeted proteolysis relates the stability of a protein to the nature of its N-terminus. The arginine (Arg) branch of the N-end rule results in exposure of Cys at the N-terminus, which can undergo *S*-nitrosation

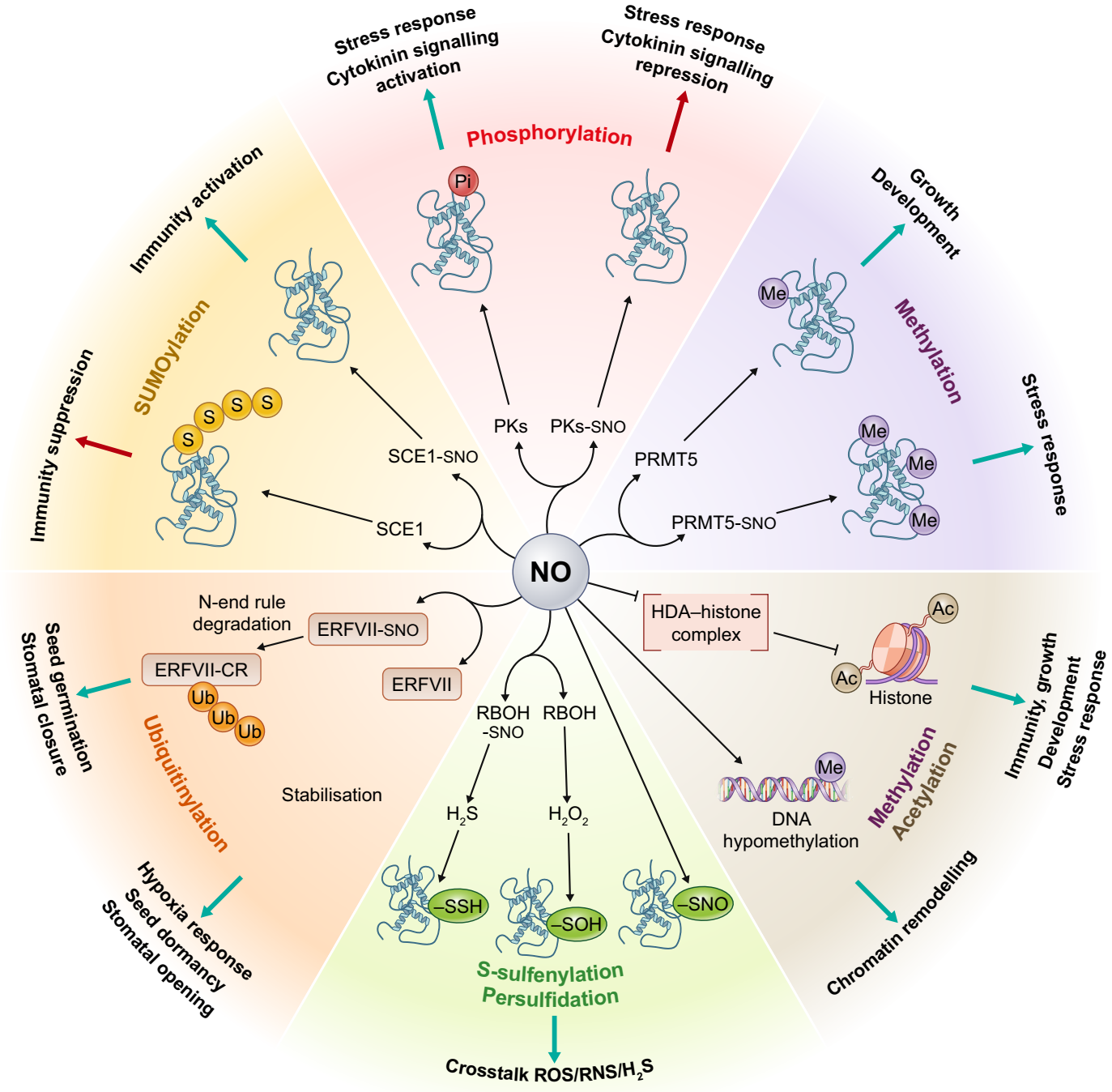

Fig. 2 Nitric oxide (NO) regulates a series of diverse post-translational modifications/signalling systems. Integrative schematic representation of cross-talk between NO and various post-translational modification/signalling systems. A major route for NO bioactivity is through protein *S*-nitrosation (SNO) to form *S*-nitrosated proteins. NO-modified regulators modulate downstream processes through diverse chemical modification systems. Chemical modifications include ubiquitinylation (Ub), SUMOylation (S), phosphorylation (Pi), methylation (Me), acetylation (Ac), S-sulfenylation (SOH) and persulfidation (SSH). Plant functions regulated by these processes are indicated at the periphery of the diagram. ERFVII, group VII ethylene response factor; ERFVII-CR, arginylated ERFVII; HAD, histone deacetylase; PKs, protein kinases; PRMT5, protein arginine methyltransferase 5; RBOH, respiratory burst oxidase homologue (NADPH oxidase); SCE1, SUMO E2 enzyme; SUMO, small ubiquitin-like modifier.

or oxidation to sulfenic or sulfonic acid, triggering arginylation of the target protein by arginyl-tRNA transferases (ATEs). These enzymes transfer Arg from Arg-tRNA to the Nt alpha-amino group of the Nt residue, leading to *N*-recognin-mediated ubiquitination and subsequent degradation (Varshavsky, 2011).

Group VII ethylene response factors (ERFs) are important regulators of oxygen sensing, as they become substrates of the N-end rule pathway. Significantly, group VII ERFs are also degraded in the presence of NO and oxygen and may thus serve as NO and oxygen sensors regulating NO function in a number of developmental processes (Gibbs *et al.*, 2014). Thus, oxygen sensing during hypoxia (reduced oxygen levels), occurring, for example, in flooded roots, also requires low levels of NO in order to stabilise group VII ERFs, which orchestrate cellular responses, ameliorating the impact of hypoxia. Under hypoxia, as the oxygen level decreases, typically, NO levels increase (Gupta *et al.*, 2005), presenting a problem. It has recently been shown that ethylene can enhance group VII ethylene response factor (ERFVII) stability before hypoxia by increasing the NO-scavenger Phytoglobin1 (Hartman *et al.*, 2019). This ethylene-mediated NO depletion and consequent ERFVII accumulation might enable preadaptation of plants before hypoxia. In summary, the emerging findings suggest that NO-dependent modification of sentinel proteins embedded within the N-end rule protein degradation pathway may under some circumstances enable NO perception, while depletion of this molecule by Phytoglobin1 supports preadaptation to hypoxia.

VII. NO regulation of methylation linked to pre-mRNA splicing

Recently, a novel mechanism of NO cross-talk with protein arginine methylation, a common post-translational modification that regulates multiple biological processes has been identified in plant stress responses (Hu *et al.*, 2017). Arginine methyltransferases (PRMTs), utilise *S*-adenosyl-L-methionine as donor of a methyl group transferred to target arginine residues. PRMTs play wide roles in the biology of the cell, including pre-mRNA splicing and mRNA translation (Blanc & Richard, 2017). Plant PRMTs are known to control key developmental processes including growth, flowering, the circadian cycle and also response to salinity (Ahmad & Cao, 2012). Among nine PRMT families, PRMT5 is localised to both the nucleus and the cytoplasm and is one of the most highly conserved and broadly expressed genes in multicellular eukaryotes. Recently, stress-induced NO-dependent *S*-nitrosation of Arabidopsis PRMT5 at Cys125 has been demonstrated, and increases the methyltransferase activity of this enzyme (Hu *et al.*, 2017). Enhanced *S*-nitrosation of PRMT5 in plants with loss-of-function mutations in *S*-nitrosoglutathione (GSNO) reductase (GSNOR) (Feechan *et al.*, 2005; Lee *et al.*, 2008b; Chen *et al.*, 2009) suggests that this enzyme is indirectly regulated by GSNOR activity, which controls global levels of GSNO, a natural NO donor. Importantly, through its effect on PRMT5 activity, NO modulates pre-mRNA splicing during plant stress. This process might represent a novel post-transcriptional mechanism by which NO diversifies the stress-induced proteome through regulation of functional transcripts and formation of new splice variants mediated by *S*-nitrosation of PRMT5 (Frungillo & Spoel, 2017). Whether *S*-nitrosation of other PRMT Cys residues, Cys260 and Cys425 (Hu *et al.*, 2017), is biologically relevant, requires further investigation, in addition to how S-nitrosation might potentially affect other PRMT5 functions in plants, that is control of circadian rhythms (Hong *et al.*, 2010). Interestingly, rat PRMT1 is also under redox control through reversible oxidation of Cys residues to sulfenic acid by H_2O_2, resulting in concentration-dependent inhibition of methyltransferase activity (Begara-Morales *et al.*, 2015). Thus, in the wider context of redox signalling, it is intriguing to speculate that plant PRMTs might also be modulated by ROS.

VIII. Conclusions

It is now becoming apparent that a major route for NO bioactivity is through the manipulation of key PTMs, for example SUMOylation, phosphorylation, persulfidation and acetylation (Fig. 2). By targeting key Cys residues, which function as regulatory redox switches, for oxidative modification, principally through *S*-nitrosation, NO is able to modulate the functions of these ubiquitous and fundamental PTMs, tailoring cellular responses to diverse challenges. The identification and subsequent characterisation of these strategically evolved redox switches will present exciting future opportunities to shape protein function towards advantageous outcomes. For example, redox switches could be designed and implemented by emerging gene editing strategies to potentially control a plethora of key biological processes underpinning a variety of important agricultural traits. The ability of NO to regulate the regulator may be at the heart of these new technologies.

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References

Ahmad A, Cao X. 2012. Plant PRMTs Broaden the scope of arginine methylation. *Journal of Genetics and Genomics* **39**: 195–208.

Aroca Á, Serna A, Gotor C, Romero LC. 2015. S-sulfhydration: a cysteine posttranslational modification in plant systems. *Plant Physiology* **168**: 334 LP–342.

Arruebarrena A, Palma D, Di LM, Salvatore SR, Martín J, Ambrosio D, García-mata C, Schopfer FJ, Laxalt AM. 2020. Nitro-oleic acid triggers ROS production via NADPH oxidase activation in plants: a pharmacological approach. *Journal of Plant Physiology* **246–247**: 153128.

Bannister AJ, Kouzarides T. 2011. Regulation of chromatin by histone modifications. *Cell Research* **21**: 381–395.

Begara-Morales JC, Sánchez-Calvo Beatriz, Chaki M, Mata-Pérez C, Valderrama R, Padilla MN, López-Jaramillo J, Luque F, Corpas FJ, Barroso JB. 2015. Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and S-nitrosylation. *Journal of Experimental Botany* **66**: 5983–5996.

Begara-Morales JC, Sánchez-Calvo Beatriz, Chaki M, Valderrama R, Mata-Pérez C, López-Jaramillo J, Padilla MN, Carreras A, Corpas FJ, Barroso JB. 2014. Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and S-nitrosylation. *Journal of Experimental Botany* **65**: 527–538.

Besson-Bard A, Courtois C, Gauthier A, Dahan J, Dobrowolska G, Jeandroz S, Pugin A, Wendehenne D. 2008a. Nitric oxide in plants: production and cross-talk with Ca2+ signaling. *Molecular Plant* **1**: 218–228.

Besson-Bard A, Pugin A, Wendehenne D. 2008b. New insights into nitric oxide signaling in plants. *Annual Review of Plant Biology* **59**: 21–39.

Blanc RS, Richard S. 2017. Arginine methylation: the coming of age. *Molecular Cell* **65**: 8–24.

Bossis G, Melchior F. 2006. SUMO: regulating the regulator. *Cell Division* **1**: 13.

van den Burg HA, Kini RK, Schuurink RC, Takken FLW. 2010. Arabidopsis small ubiquitin-like modifier Paralogs have distinct functions in development and defense. *Plant Cell* **22**: doi: 10.1105/tpc.109.070961

Chen R, Sun S, Wang C, Li Y, Liang Y, An F, Li C, Dong H, Yang X, Zhang J *et al.* 2009. The Arabidopsis PARAQUAT RESISTANT2 gene encodes an S-nitrosoglutathione reductase that is a key regulator of cell death. *Cell Research* **19**: 1377–1387.

Corpas FJ, González-Gordo S, Cañas A, Palma JM. 2019. Nitric oxide and hydrogen sulfide in plants: which comes first? *Journal of Experimental Botany* **70**: 4391–4404.

Corpas FJ, Palma JM, del Río LA, Barroso JB. 2013. Protein tyrosine nitration in higher plants grown under natural and stress conditions. *Frontiers in Plant Science* **4**: 29.

Del Castello F, Nejamkin A, Cassia R, Correa-Aragunde N, Fernández B, Foresi N, Lombardo C, Ramirez L, Lamattina L. 2019. The era of nitric oxide in plant biology: twenty years tying up loose ends. *Nitric Oxide* **85**: 17–27.

Dissmeyer N, Rivas S, Graciet E. 2018. Life and death of proteins after protease cleavage: protein degradation by the N-end rule pathway. *New Phytologist* **218**: 929–935.

Feechan A, Kwon E, Yun B-W, Wang Y, Pallas JA, Loake GJ. 2005. A central role for S-nitrosothiols in plant disease resistance. *Proceedings of the National Academy of Sciences, USA* **102**: 8054–8059.

Feng J, Wang C, Chen Q, Chen H, Ren B, Li X, Zuo J. 2013. S-nitrosylation of phosphotransfer proteins represses cytokinin signaling. *Nature Communications* **4**: 1529.

Frederickson Matika DE, Loake GJ. 2014. Redox regulation in plant immune function. *Antioxidants & Redox Signaling* **21**: 1373–1388.

Frungillo L, Spoel SH. 2017. Preview modulating the modulator: regulation of protein methylation by nitric oxide. *Molecular Cell* **67**: 535–537.

Gibbs DJ, Bacardit J, Bachmair A, Holdsworth MJ. 2014. The eukaryotic N-end rule pathway: conserved mechanisms and diverse functions. *Trends in Cell Biology* **24**: 603–611.

Gibbs DJ, Bailey M, Tedds HM, Holdsworth MJ. 2016. From start to finish: amino-terminal protein modifications as degradation signals in plants. *New Phytologist* **211**: 1188–1194.

Gupta KJ, Stoimenova M, Kaiser WM. 2005. In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, *in vitro* and *in situ*. *Journal of Experimental Botany* **56**: 2601–2609.

Hancock JT. 2019. Considerations of the importance of redox state for reactive nitrogen species action. *Journal of Experimental Botany* **70**: 4323–4331.

Hartman S, Liu Z, van Veen H, Vicente J, Reinen E, Martopawiro S, Zhang H, van Dongen N, Bosman F, Bassel GW *et al.* 2019. Ethylene-mediated nitric oxide depletion pre-adapts plants to hypoxia stress. *Nature Communications* **10**: 4020.

Hong S, Song H-R, Lutz K, Kerstetter RA, Michael TP, McClung CR. 2010. Type II protein arginine methyltransferase 5 (PRMT5) is required for circadian period determination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **107**: 21211–21216.

Hu J, Yang H, Mu J, Lu T, Peng J, Deng X, Kong Z, Bao S, Cao X, Zuo J. 2017. Nitric oxide regulates protein methylation during stress responses in plants. *Molecular Cell* **67**: 702–710.e4.

Huang J, Willems P, Wei B, Tian C, Ferreira RB, Bodra N, Martínez Gache SA, Wahni K, Liu K, Vertommen D *et al.* 2019. Mining for protein S-sulfenylation in Arabidopsis uncovers redox-sensitive sites. *Proceedings of the National Academy of Sciences, USA* **116**: 21256–21261.

Hussain A, Mun B, Imran QM, Lee S, Adamu TA, Kim K, Yun B-W. 2016. Nitric oxide mediated transcriptome profiling reveals activation of multiple regulatory pathways in *Arabidopsis thaliana*. *Frontiers in Plant Science* **7**: 975.

Kelley EE, Batthyany CI, Hundley NJ, Woodcock SR, Bonacci G, Del Rio JM, Schopfer FJ, Lancaster JR, Freeman BA, Tarpey MM. 2008. Nitro-oleic acid, a novel and irreversible inhibitor of xanthine oxidoreductase. *Journal of Biologcal Chemistry* **283**: 36176–36184.

Kolbert Z, Feigl G. 2017. Cross-talk of reactive oxygen species and nitric oxide in various processes of plant development. In: Singh VP, Singh S, Tripathi DK, Prasad SM, Chauhan D eds. *Reactive oxygen species in plants*. New Jersey, USA: John Wiley & Sons, 261–289.

Kovacs I, Holzmeister C, Wirtz M, Geerlof A, Fröhlich T, Römling G, Kuruthukulangarakoola GT, Linster E, Hell R, Arnold GJ *et al.* 2016. ROS-mediated inhibition of S-nitrosoglutathione reductase contributes to the activation of anti-oxidative mechanisms. *Frontiers in Plant Science* **7**: 1–17.

Lee J, Lee Y, Lee MJ, Park E, Kang SH, Chung CH, Lee KH, Kim K. 2008a. Dual modification of BMAL1 by SUMO2/3 and ubiquitin promotes circadian activation of the CLOCK/BMAL1 complex. *Molecular and Cellular Biology* **28**: 6056–6065.

Lee U, Wie C, Fernandez BO, Feelisch M, Vierling E. 2008b. Modulation of nitrosative stress by S-nitrosoglutathione reductase is critical for thermotolerance and plant growth in Arabidopsis. *Plant Cell* **20**: 786–802.

Leterrier M, Chaki M, Airaki M, Valderrama R, Palma JM, Barroso JB, Corpas FJ. 2011. Function of S-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress. *Plant Signaling & Behavior* **6**: 789–793.

Lindermayr C, Saalbach G, Durner J. 2005. Proteomic identification of S-nitrosylated proteins. *Plant Physiology* **137**: 921–930.

Ling T, Vandelle E, Bellin D, Kleinfelder-Fontanesi K, Huang JJ, Chen J, Digby AM, Delledonne M. 2012. Nitric oxide produced during the hypersensitive response modulates the plant signaling network and inhibits the pathogen's virulence machinery. *Nitric Oxide* **27**: S9.

Lozano-Juste J, Colom-Moreno R, León J. 2011. In vivo protein tyrosine nitration in *Arabidopsis thaliana*. *Journal of Experimental Botany* **62**: 3501–3517.

Mengel A, Ageeva A, Georgii E, Bernhardt J, Wu K, Durner J, Lindermayr C. 2017. Nitric oxide modulates histone acetylation at stress genes by inhibition of histone deacetylases. *Plant Physiology* **173**: 1434–1452.

Minguez P, Parca L, Diella F, Mende DR, Kumar R, Helmer-Citterich M, Gavin A-C, van Noort V, Bork P. 2012. Deciphering a global network of functionally associated post-translational modifications. *Molecular Systems Biology* **8**: 599.

Nakamura T, Lipton SA. 2019. Nitric oxide-dependent protein post-translational modifications impair mitochondrial function and metabolism to contribute to neurodegenerative diseases. *Antioxidants & Redox Signaling*. **32**: 7916.

Ortega-Galisteo AP, Gupta DK, Pazmiño DM, Sandalio LM, Rodríguez-Serrano M, Romero-Puertas MC. 2012. S-Nitrosylated proteins in pea (*Pisum sativum* L.) leaf peroxisomes: changes under abiotic stress. *Journal of Experimental Botany* **63**: 2089–2103.

Ou X, Zhuang T, Yin W, Miao Y, Wang B, Zhang Y, Lin X, Xu C, von Wettstein D, Rustgi S *et al.* 2015. DNA methylation changes induced in rice by exposure to high concentrations of the nitric oxide modulator, sodium nitroprusside. *Plant Molecular Biology Reporter* **33**: 1428–1440.

Saleh A, Withers J, Mohan R, Marqués J, Gu Y, Yan S, Zavaliev R, Nomoto M, Tada Y, Dong X. 2015. Posttranslational modifications of the master transcriptional regulator NPR1 enable dynamic but tight control of plant immune responses. *Cell Host & Microbe* **18**: 169–182.

Servet C, Conde N, Zhou D. 2010. Histone acetyltransferase AtGCN5/HAG1 is a versatile regulator of developmental and inducible gene expression in Arabidopsis. *Molecular Plant* **3**: 670–677.

Shen J, Zhang J, Zhou M, Zhou H, Cui B, Gotor C, Romero LC, Fu L, Yang J, Foyer CH *et al.* 2020. Persulfidation-based modification of cysteine desulfhydrase and the NADPH oxidase RBOHD controls guard cell abscisic acid signaling. *Plant Cell* **32**: 1000–1017.

Shen Y, Wei W, Zhou D-X. 2015. Histone acetylation enzymes coordinate metabolism and gene expression. *Trends in Plant Science* **20**: 614–621.

Shi H, Ye T, Zhu J-K, Chan Z. 2014. Constitutive production of nitric oxide leads to enhanced drought stress resistance and extensive transcriptional reprogramming in Arabidopsis. *Journal of Experimental Botany* **65**: 4119–4131.

Skelly MJ, Malik SI, Le Bihan T, Bo Y, Jiang J, Spoel SH, Loake GJ. 2019. A role for S-nitrosylation of the SUMO-conjugating enzyme SCE1 in plant immunity. *Proceedings of the National Academy of Sciences, USA* **116**: 17090–17095.

Tompa P, Davey NE, Gibson TJ, Babu MM. 2014. A million peptide motifs for the molecular biologist. *Molecular Cell* **55**: 161–169.

Varshavsky A. 2011. The N-end rule pathway and regulation by proteolysis. *Protein Science* **20**: 1298–1345.

Wawer I, Bucholc M, Astier J, Anielska-Mazur A, Dahan J, Kulik A, Wysłouch-Cieszynska A, Zaręba-Kozioł M, Krzywinska E, Dadlez M *et al.* 2010. Regulation of Nicotiana tabacum osmotic stress-activated protein kinase and its cellular partner GAPDH by nitric oxide in response to salinity. *Biochemical Journal* **429**: 73–83.

Yang H, Mu J, Chen L, Feng J, Hu J, Li L, Zhou J-M, Zuo J. 2015. S-nitrosylation positively regulates ascorbate peroxidase activity during plant stress responses. *Plant Physiology* **167**: 1604–1615.

Yun B, Skelly MJ, Yin M, Yu M, Mun B, Lee S, Hussain A, Spoel SH, Loake GJ. 2016. Nitric oxide and S -nitrosoglutathione function additively during plant immunity. *New Phytologist* **211**: 516–526.

Yun B-W, Feechan A, Yin M, Saidi NBB, Le Bihan T, Yu M, Moore JW, Kang J-G, Kwon E, Spoel SH *et al.* 2011. S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. *Nature* **478**: 264–268.