Recent advances in the regulation of plant immunity by S-nitrosylation

RECENT ADVANCES IN THE REGULATION OF PLANT IMMUNITY BY *S*-NITROSYLATION

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Highlights

Herein we provide an update on recent developments of *S*-nitrosylation in plant immune function which includes: both how this redox-based post-translational modification controls key aspects of immunity and also how *S*-nitrosylation can disable aspects of pathogen virulence.

Abstract

S-nitrosylation, the addition of a nitric oxide (NO) moiety to a reactive protein cysteine (Cys) thiol, to form a protein *S*-nitrosothiol (SNO), is emerging as a key regulatory post-translational modification (PTM) to control the plant immune response. NO also *S*-nitrosylates the antioxidant tripeptide, glutathione (GSH), to form *S*-nitrosoglutathione (GSNO), both a storage reservoir of NO bioactivity and a natural NO donor. GSNO and by extension, *S*-nitrosylation, is controlled by GSNO reductase1 (GSNOR1). The emerging data suggests that GSNOR1 itself is a target of NO-mediated *S*-nitrosylation, which subsequently controls its selective autophagy, regulating cellular protein SNO levels. Recent findings also suggest that *S*-nitrosylation may be deployed by pathogen-challenged host cells to counteract the effect of delivered microbial effector proteins, that promote pathogenesis and by the pathogens themselves to augment virulence. Significantly, it also appears that *S*-nitrosylation may regulate plant immune functions by controlling SUMOylation, a peptide-based PTM. In this context, global SUMOylation is regulated by *S*-nitrosylation of SUMO conjugating enzyme, SCE1 at Cys 139. This redox-based PTM has also been shown to control the function of a key zinc finger transcriptional regulator during the establishment of plant immunity and the selective degradation of GSNOR1 by autophagy. Here, we provide an update of these recent advances.

Introduction

A key feature of the plant immune response is the associated striking changes in cellular redox status resulting from elevated levels of reactive oxygen species~~intermediates~~ (ROS~~Is~~) and reactive nitrogen species (RNS) (Couturier *et al.*, 2013; Skelly and Loake, 2013; Yu *et al.*, 2014). RNS comprise predominately nitric oxide (NO), the NO radical ($NO^{\cdot}$) and other derivatives including, peroxynitrite ($ONOO^{-}$), dinitrogen trioxide (N_2O_3), and nitrogen dioxide (NO_2) (Corcoran and Cotter, 2013; Grant and Loake, 2000; Couturier *et al.*, 2013). These small, redox-active molecules, function in redox signalling networks helping to orchestrate the plant response to a plethora of potentially stresses (Yu *et al.*, 2012; Couturier *et al.*, 2013; Lisjak *et al.*, 2013; Turkan, 2017).

NO is thought to be produced majorly from the reduction of nitrate~~s~~ / nitrite by NADPH and pH dependent nitrate reductase (NR) (Rockel *et al.*, 2002; Bolwell and Daudi, 2009; Sanz *et al.*, 2015; Astier *et al.*, 2018). However, other potential sources include: mitochondria, through the electron transport chain, peroxisomes and chloroplasts (Kolbert *et al.*, 2019). In *Arabidopsis*, NR is located in the cytosol and encoded by two structural genes *NIA1* and *NIA2*, with *NIA2* exhibiting the highest observed nitrate reductase activity (Wilkinson and Crawford, 1991; Frederickson Matika and Loake, 2014). NO bioavailability is tightly regulated by scavenging mechanisms (Chamizo-Ampudia *et al.*, 2017). Thus, NO reacts with freely available intracellular glutathione to form *S*-nitrosoglutathione (GSNO) that acts as both a storage reservoir and a natural donor of NO. GSNO is metabolized by a cytosolic enzyme *S*-nitrosoglutathione reductase 1 (GSNOR1) to glutathione disulphide and ammonia (Feechan *et al.*, 2005~~a~~; Yun *et al.*, 2011, 2016). NO can also be scavenged by ROS ~~reactive oxygen species (ROS)~~ such as superoxides (O_2^{-}) to form ONOO− (Romero-Puertas *et al.*, 2007; Gaupels *et al.*, 2011; Begara-Morales *et al.*, 2014). NO can also be consumed by p~~P~~lant hemoglobins (Hb) which ~~. These~~ are ubiquitous proteins conserved across biological kingdoms ~~(Gardner, 2012)~~ and have ~~also~~ been shown to scavenge NO by catalyzing the dioxygenation of NO to form nitrate~~s~~ (Gardner, 2012; Chamizo-Ampudia *et al.*, 2017).

Generated NO functions majorly through *S*-nitrosylation, a prototypic, redox-based post-translational modification (PTM). This process involves the addition of an NO moiety to a rare, highly reactive cysteine (Cys) thiol to form an *S*-nitrosothiol (SNO) in a fashion akin to more established PTMs, such as phosphorylation (Spadaro *et al.*, 2010; Friso and van Wijk, 2015; Gupta *et al.*, 2020). Subsequently, *S*-nitrosylation may regulate protein localization, protein function, protein-protein interactions and protein stability in an allosteric-like fashion following associated conformational changes (Astier *et al.*, 2012*b*; Vanzo *et al.*, 2014). To date, a number of plant proteins integral to plant immunity have been found to be *S*-nitrosylated, including: NADPH oxidase (Yun *et al.*, 2011, 2016), NPR1 (Tada et al., 2008; Spoel and Loake, 2011), TGA1 (Lindermayr *et al.*, 2010), AtSABP3 (Wang *et al.*, 2009) and Peroxyredoxin (Romero-Puertas 2007).

In this review, we highlight recent advances associated with protein *S*-nitrosylation during plant immunity. We discuss host-driven *S*-nitrosylation targeting pathogen derived effector proteins delivered to the inside of plant cells, subsequently disabling their function(s) (Ling *et al.*, 2017). Further, we outline the ability of *S*-nitrosylation to control the transcriptional reprogramming of plant gene expression during the immune response (Cui *et al.*, 2018). We also review the role of *S*-nitrosylation in promoting autophagy, by destabilizing immune-related proteins leading to lysosome degradation promoting pathogen susceptibility (Zhan *et al.*, 2018). Lastly, we discuss the regulation of SUMOylation by *S*-nitrosylation (Skelly *et al.*, 2019) during the plant immune response.

***S*-nitrosylation exploited by the host and pathogen during immuno responses**

Plant pathogens are invading microorganisms aimed to derive nutrients from the host to support their growth and development (Davidsson *et al.*, 2013; Snelders *et al.*, 2018). To achieve this pathogens secretes effectors which are the proteinaceous molecules released to circumvent the host defence machinery (Kamoun, 2007). They are grouped majorly into two

116 categories, the apoplastic effectors that act outside the cell and the cytoplasmic effectors that
117 act inside the host cell (Asai and Shirasu, 2015). In fungi and oomycetes, effectors are
118 produced and released by haustoria, which also function as intracellular feeding structures
119 (Wang *et al.*, 2017). In bacterial pathogens, effector proteins are secreted via six secretory
120 systems (Setti *et al.*, 2014). For instance, the type two secretory system (T2SS) also known
121 as sec dependent system release the apoplastic effectors, cell wall degrading enzymes and
122 other hydrolytic enzymes such as cellulases, xylanases, amylases and proteases (Setti *et al.*,
123 2014; Pfeilmeier *et al.*, 2016). Whereas the type three secretory system (T3SS) is the
124 molecular machinery that delivers cytoplasmic effectors into host cells. This system is encoded
125 by *Hypersensitive Response* and *Pathogenicity* (*HRP*) genes (Setti *et al.*, 2014). The
126 transferred effectors promote pathogenicity on a susceptible host and a hypersensitive
127 response on a resistant host by interfering with key cellular processes (Buttner and He, 2009).
128
129
130 Although the molecular mechanisms underpinning the release of bacterial plant pathogen
131 effectors into the apoplast or cytosol has been largely elucidated, the identification of their
132 possible host protein targets is still continuing at pace (Park *et al.*, 2012; Hann *et al.*, 2014; Liu
133 *et al.*, 2016; Wang *et al.*, 2017). As previously stated, effector-triggered immunity (ETI) results
134 in a conspicuous nitrosative burst, although the source(s) of NO still remain controversial
135 (Schlicht and Kombrink, 2013). During the onset of ETI in *Arabidopsis* the bacterial effector
136 protein HopAI1 (HRP-dependent outer protein A1) of *Pseudomonas syringae pv tomato*
137 DC3000 (*Pst*DC3000) is *S*-nitrosylated inhibiting the phosphothreonine lyase activity of this
138 effector (Ling *et al.*, 2017). HopAI1 is known to enhances *Pst* strain 0288-9 virulence in tomato
139 plants. In a similar fashion, the transgenic expression of HopAI1 in *Arabidopsis* increases
140 susceptibility to *Pst*DC3000 by compromising ETI (Zhang *et al.*, 2007).
141
142 HopAI1 targets and suppresses mitogen activated protein kinases (MAPKs) activated by
143 exposure to pathogen-associated molecular patterns (PAMPS) such as flagellin 22 (Bolwell

and Daudi, 2009). MAPKs are involved in signal transduction mediated by phosphorylation of proteins leading to activation of defence genes that orchestrate the development of a plethora of immune-related functions (Taj *et al.*, 2010). The canonical pathway for MAPK signaling is a phosphorylation cascade supported by associated mitogen activated protein kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK) through to MAPK function (Taj *et al.*, 2010). Finally the activated MAPK is transported to the nucleus where it typically phosphorylates one or more target transcription factors, supporting transcriptional reprogramming (Taj *et al.*, 2010). The HopAI1 effector is thought to act by removing a phosphate group from phosphothreonine required for HopAI1 activity (Zhang *et al.*, 2007). *S*-nitrosylation of HopAI1 occurs at Cys 138, a non-catalytic residue, restoring MAPK signalling and consequently, disarming a key pathogen infection strategy (Ling *et al.*, 2017). In addition, overexpression of HopAI1 Cys138S (Cys is replaced with Serine), an *S*-nitrosylation insensitive mutant, triggered autoimmunity and failed to promote the anticipated pathogen susceptibility (Ling *et al.*, 2017).

On the other hand, *S*-nitrosylation can be utilized by the pathogen to enhance virulence. For instance, cell division control protein 48 (CDC48) also known as p97 or vVal~~s~~os~~l~~in containing protein (VCP) is an evolutionally conserved ATPase of AAA family present in yeast, plants and animals (Baek *et al.*, 2013; Bodnar *et al.*, 2018). CDC48 is involved in a number of growth and development processes including vesicle trafficking, mitochondrial and proteasomal degradation of ubiquitinated proteins (Taylor and Rutter, 2011; Barthelme and Sauer, 2013). In *Arabidopsis*, it localizes across a number of subcellular components including the plasma membrane, cytoplasm, endoplasmic reticulum and nucleus (Aker *et al.*, 2006; Gallois *et al.*, 2013). Previous studies in *Drosophila* and *Arabidopsis* have shown that CDC48 undergoes *S*-nitrosylation at conserved cysteine sites inhibiting the ATP hydrolysis activity (Noguchi *et al.*, 2005; Fares *et al.*, 2011). Recently a similar event has been elucidated in *Nicotiana tabacum* during interaction with *Phytophthora cryptogea*, an oomycete pathogen infecting several ornamental plants (Rosnoblet *et al.*, 2017). The pathogen produces several halo proteins known as elicitins that act as avirulence factors in *Phytophthora cryptogea*-tobacco

interactions and function as *Phytophthora* pathogenicity factors on other plants (Panabières and Le Berre, 1999). The cCryptogein protein is one of the elicitors produced from *Phytophthora cryptogea* during interaction with tobacco and has been shown to induce the *S*-nitrosylation of NtCDC48 at Cys 526 *in vivo* in *Nicotiana* cells compromising immune responses (Astier *et al.*, 2012*a*; Rosnoblet *et al.*, 2017). Thus, *S*-nitrosylation may be exploited by both hosts and pathogens to promote either immunity or virulence, respectively.

_S_-nitrosylation regulates the zinc finger transcription factor, SRG1

By applying global expression profiling techniques, Cui *et al.*, (2018) uncovered a gene termed, *S-nitrosothiol regulated gene 1* (*SRG1*), which belonged to C2H2 zinc finger containing transcriptional factor (TF) family that was exquisitely sensitive to transcriptional regulation by NO. Further, accrual of SRG1 transcripts also occurred following pathogen challenge. The C2H2 zinc finger domain is ubiquitous in higher eukaryotes. This domain consists of 28-30 amino acid residues arranged in β and α sheets. Structure stabilization is achieved by two conserved Cys of the β sheet and two conserved histidine at the C-terminus of the α sheet coordinating a zinc atom (Fedotova *et al.*, 2017). Transgenic *Arabidopsis* plants over expressing *SRG1* have increased hypersensitive response like cell death, increased ROS accumulation, increased *Pathogenesis Related 1* (*PR1*) gene expression and enhanced resistance to *Pst*DC3000 bacteria. In addition loss-of-function mutants of *SRG1* have increased susceptibility to *Pst*DC3000 (Cui *et al.*, 2018). These results suggests that SRG1 is a positive regulator of plant immunity. However, SRG1 possess an ETHYLENE RESPONSE FACTOR associated amphiphilic repression (EAR) domain located at the C-terminus and this enables an interaction with the corepressor, TOPLESS, to form a transcriptional repressor complex (Martin-arevalillo *et al.*, 2017; Cui *et al.*, 2018). The transcriptional repression activity of SRG1 was validated *in vivo* in *Arabidopsis* protoplasts where SRG1 was found to reduce the expression of *Luciferase* (*LUC*) reporter gene to ≈50% (Cui *et al.*, 2018). Hence, SRG1 appears to function as a transcription repressor. To function as an activator of plant immunity, SRG1 therefore presumably must repress the activity of one or more repressors.

SRG1 exhibits reduced repressor activity in the *Arabidopsis thaliana S-nitrosoglutathione reductase* (*atgnor1-3*) mutant relative to wild-type Col-0. The *atgsnor1-3* line has increased levels of *S*-nitrosoglutathione (GSNO), a natural NO donor. Thus, GSNOR1 controls the global bioavailability of GSNO (Feechan *et al.*, 2005~~a~~; Umbreen *et al.*, 2018, 2019). This suggests that the repressor activity of SRG1 is blunted by GSNO accumulation. Indeed, *S*-nitrosylation of SRG1 at Cys 87 was found to attenuate binding of this transcriptional repressor at the DNA motif AG/CT. This negatively regulated plant immunity (Fukushima *et al.*, 2012; Cui *et al.*, 2018). *S*-nitrosylation of SRG1 is thought to drive the abrogation of the zinc finger structure required for the functional activity of zinc finger proteins (Chasapis *et al.*, 2010). In mammalian systems, it is well established that NO can regulate the C2H2 zinc finger structure (Kroncke and Carlberg, 2000; Sha and Marshall, 2012), but the underpinning molecular mechanism has not been elucidated.

Collectively, these data suggest a model whereby following pathogen recognition and the activation of a nitrosative burst, leading to NO accrual, *SRG1* transcription is activated. Subsequently, SRG1 binds to the target promoter of one or more immune repressors possessing an AG/CT motif, suppressing the function of the cognate gene(s). This contributes to the activation of plant immunity. Subsequently, as the NO concentration increases during the later stages of the defence response, SRG1 becomes *S*-nitrosylated at Cys87 inhibiting its DNA binding activity and by extension, relieving the suppression of one or more immune repressors. The expression of these repressors may then contribute to the attenuation of the transient immune response. Thus, SRG1 function enables both the activation and subsequent repression of plant immunity and this switch is regulated by the *S*-nitrosylation of this C2H2 zinc finger transcription factor.

_S_-nitrosylation targets GSNOR for autophagy

Autophagy is a major catabolic route in eukaryotes degrading not only proteins but also entire organelle complexes (Klionsky and Codogno, 2013). The autophagy process is initiated when the lipid bilayer phagophore contributed by the endoplasmic reticulum and / or *trans*-Golgi apparatus engulf the intracellular cytoplasmic cargo leading to the formation of an autophagosome. The autophagosome fuses with the vacuole degrading the delivered materials. The products of degradation are then recycled for the construction of macro-molecules (Glick *et al.*, 2010; Sieńko *et al.*, 2020; Stefaniak *et al.*, 2020). A number of autophagy related protein genes (ATG) play significant roles in autophagosome formation, but the two ubiquitination-like system of ATG8 lipidation and the ATG12 protein conjugation system are especially well investigated (Yoshimoto *et al.*, 2010; Yoshimoto and Ohsumi, 2018). In the ATG8 lipidation system, the C-terminal extension of ATG8 is processed by a Cys protease, ATG4, exposing the glycine residue. The truncated ATG8 is activated by an E1 enzyme, ATG7, via the formation of a thioester bond between the glycine residue of ATG8 and the Cys residue of ATG7. ATG8 is then transferred to a Cys residue of an E2 enzyme ATG3 and finally conjugated to a phospholipid phosphatidylethanolamine (PE) head group, leading to the formation of an autophagosome (Ichimura *et al.*, 2000). In the ATG12 conjugation system, ATG12 is activated by ATG7 and transferred to the Cys residue of an E2 like enzyme, ATG10. ATG12 is finally conjugated on to ATG5, via isopeptide bond formation between the lysine residue of ATG5 and the glycine residue of ATG12. The ATG12-ATG5 complex is subsequently able to mediate autophagosome formation (Mizushima *et al.*, 1998; Yoshimoto *et al.*, 2010; Yoshimoto and Ohsumi, 2018).

S-nitrosylation has been shown to control the availability of GSNOR1 by targeting it to autophagy pathway (Zhan *et al.*, 2018). GSNOR1 is a master regulator of intracellular levels of NO and proteome wide *S*-nitrosylation (Feechan *et al.*, 2005~~*b*~~). Previous reports have shown that GSNOR1 can be *S*-nitrosylated constraining its de-nitrosylation functions achieved by scavenging GSNO (Frungillo *et al.*, 2014). Recently, additional data has emerged,

confirming the *S*-nitrosylation of this key redox regulatory enzyme (Zhan *et al.*, 2018). *S*-nitrosylation of GSNOR1 has been shown to occur predominantly at Cys 10 and 370. *In vitro* experiments in the presence of NO donors and overexpression of GSNOR1 in *Arabidopsis NO overexpressor1* (*nox1*) mutants, which exhibit elevated levels of NO, resulted in the instability of GSNOR1 (Frungillo *et al.*, 2014). The degradation of GSNOR1 is attributed to structural modification mediated by *S*-nitrosylation. Autophagy related lysosomes were demonstrated as the final destination of *S*-nitrosylated GSNOR1. Proteasome inhibitors did not enhance the accumulation of *S*-nitrosylated GSNOR1, while autophagy inhibitors significantly elevated the levels of this enzyme. Thus, *S*-nitrosylated GSNOR1 is targeted to the autophagy pathway rather than the proteasome pathway. Moreover, elevated levels of GSNOR1 were detected in autophagosomes and in *atg2*, *atg5*, *atg7* and *atg10* mutant plants (Zhan *et al.*, 2018).

Selective autophagy is induced by the intermolecular β sheet interaction between ATG8 and the ATG8 interacting motif (AIM) of the substrate (Liu and Bassham, 2012; Michaeli *et al.*, 2016). The AIM ~~motif~~ in GSNOR1 is located in a β sheet between residues 152-155. The motif is located in a pocket close to Cys 10. *S*-nitrosylation of GSNOR1 occurs at Cys 10 and Cys 370, respectively. Though the functional role of SNO formation at Cys 370 is not elucidated, the data suggests that t~~T~~he *S*-nitrosylation of GSNOR1 at Cys 10 introduces a conformation change in protein ~~the~~ structure promoting the interaction of ATG8 and GSNOR1 (Zhan *et al.*, 2018). This ~~Such inte~~interaction ~~raction~~ results in ~~to~~ GSNOR1 being directed into ~~Though the functional role of Cys 370 is not elucidated, the data suggests that *S*-nitrosylation of GSNOR1 at Cys 10 increases interactions with the~~ the autophagy pathway leading to GSNOR ~~lysosome~~ degradation. Although autophagic negative regulation of GSNOR1 mediated by *S*-nitrosylation could enhance the adaptability of plants to hypoxia (Zhan *et al.*, 2018), the loss of *GSNOR1* in *Arabidopsis* is associated with significant immune defects (Feechan *et al.*, 2005~~a~~; Chen *et al.*, 2009; Yu *et al.*, 2012; Arasimowicz and Floryszak, 2016). Overall this information suggests that excessive autophagy-dependent degradation of GSNOR1-SNO would promote the

virulence of both adapted and non-pathogens (Figure 1). Perhaps the function of this cellular GSNOR degradation is to help maintain a supply of “fresh” GSNOR1, ensuring effective GSNOR1 activity (Feechan *et al.*, 2005~~a~~; Chen *et al.*, 2009; Yu *et al.*, 2012; Arasimowicz and Floryszak, 2016; Zhan *et al.*, 2018).

S-Nitrosylation regulates SUMOylation during plant immunity

SUMOylation, a reversible post-translational modification, occurring via the covalent attachment of the small ubiquitin-like modifier (SUMO) to target proteins through an ATP dependent reaction involving activation (E1), conjugation (E2) and ligation (E3) enzymes. SUMOylation is biochemically similar to ubiquitination but functionally distinct. SUMO is conserved from yeast to humans and the SUMO pathway is essential for the survival of eukaryotic cells (Nacerddine *et al.*, 2005; Saracco *et al.*, 2007; Zhao, 2018). In animals, SUMOylation has been studied extensively and shown to regulate numerous biological functions including various human diseases such as mental disorders, strokes, cancers, neurodegenerative and heart diseases (Coppola *et al.*, 2009; Mun *et al.*, 2016; Anderson *et al.*, 2017; Liu *et al.*, 2017; Thomas and Yang, 2017).

In plants, SUMOylation has been reported to regulate immunity via the deployment of host defence responses. In tomato, T-SUMO (Homologue of human SUMO1) protein interacts with EIX (ethylene inducing xylanase) of the fungus *Trichoderma viridae* which resulted in rapid induction of defence responses by production of ethylene (Hanania *et al.*, 1999). Pathogen triggered cell death is suppressed when T-SUMO was over-expressed and anti-sense lines displayed reverse effects, suggesting SUMO negatively regulates plant immunity in tomato (Hanania *et al.*, 1999). *Arabidopsis thaliana* SAP and MIZ1 (AtSIZ1) currently the only E3 ligase identified in *Arabidopsis*, was reported to downregulate plant immunity, as an *AtSIZ1* loss-of-function mutation produced higher levels of salicylic acid (SA) with increased expression of *PR1* and hence increased resistance to *Pst*DC3000 (Lee *et al.*, 2007). Similarly, a knock-down mutant for SUMO1/2 which has reduced levels of global SUMOylation showed

312 a 10-fold increase in *PR1* gene expression and increased resistance to *Pst*DC3000 (van den
313 Burg *et al.*, 2010). Interestingly, the master regulator of SA signalling, NPR1
314 (NONEXPRESSOR OF PATHOGENESIS RELATED 1) acts as a SUMO substrate. NPR1
315 stability and binding with TFs is mediated by its SUMOylation, which is required for plant
316 immunity (Saleh *et al.*, 2015). Interestingly, recent studies have suggested that *S*-nitrosylation
317 regulates global 1/2 SUMOylation, controlling plant immunity, by directly modifying the SUMO
318 E2 enzyme, SUMO Conjugating Enzyme 1 (SCE1), at Cys 139 (Skelly *et al.*, 2019; Gupta *et*
319 *al.*, 2020). SUMO1/2 SUMOylation is thought to contribute to the negative regulation of plant
320 immunity, maintaining the repression of plant defence responses in the absence of attempted
321 pathogen infection (Skelly *et al.*, 2019). However, following pathogen challenge, a nitrosative
322 burst leads to increased levels of NO accumulation, driving *S*-nitrosylation of SCE1 at Cys139.
323 SNO formation at this Cys residue disables the E2 activity of SCE1 and thereby decreases
324 global SUMOylation levels enabling the synthesis of the immune activator, SA and the
325 activation of SA-dependent immune responses (Skelly *et al.*, 2019). Correspondingly, SCE1
326 Cys139S mutants, which are insensitive to *S*-nitrosylation, showed higher levels of SUMO
327 conjugates and increased disease susceptibility against *Pst*DC3000. These plant lines
328 exhibited reduced levels of endogenous SA accumulation following pathogen challenge but
329 respond to SA in a similar fashion to wild-type plants: i.e. strong induction of SA-dependent
330 genes upon exogenous SA application (Skelly *et al.*, 2019). Thus, *S*-nitrosylation of SCE1 at
331 Cys139 may enable SA accumulation, which subsequently supports the deployment of SA-
332 dependent defence responses (Figure 2).
333
334 Importantly, SUMOylation was previously thought to be regulated at the local level with the
335 SUMO modification of individual substrates controlled. These findings establish a parallel and
336 complementary paradigm, suggesting that SUMOylation can also be regulated at a global
337 level, by the control of SCE1 by *S*-nitrosylation. Significantly, the function of the human
338 homologue of SCE1, UBC9, was also found to be compromised by *S*-nitrosylation at the

evolutionary conserved Cys139 residue. Therefore, SNO formation at Cys139 of SCE1/UBC9 may regulate SUMOylation across phylogenetic kingdoms.

Future perspectives

Microbial pathogens deliver a plethora of effector proteins to the inside of plant cells to support pathogenesis. The work of Ling and coworkers establishes for the first time that challenged plant host cells can deploy *S*-nitrosylation to disable the function of effectors. Many pathogen effector proteins are rich in Cys residues and presumably, some of these must be solvent exposed, potentially providing target sites for *S*-nitrosylation. It will therefore be informative to determine if other effectors are disabled by this redox-based PTM. In a similar fashion, are other proteins, in addition to GSNOR, targeted by SNO formation for autophagy? Critically, are autophagy mutants disrupted in (S)NO homeostasis? Finally, it will be informative to explore if *S*-nitrosylation regulates other aspects of SUMOylation. It is becoming increasingly clear that *S*-nitrosylation is a key regulator of a plethora of molecular features underpinning the establishment of plant immunity.

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Figure legends

Figure 1. Conformational changes in GSNOR1 by *S*-nitrosylation mediates its autophagy. Generated NO reacts with glutathione (GSH) to form GSNO. GSNOR1 reduces GSNO to oxidized GSH (GSSG) and ammonia. GSSG is further reduced to GSH by GSH reductase (GR). Changes in GSNOR1 activity regulates protein-SNO by modulating GSNO levels. **(B)** Free NO *S*-nitrosylates GSNOR1 at Cys10. The *S*-nitrosylation of GSNOR1 results in conformation changes which enhances the interaction with autophagy-related protein 8 (ATG8) through its ATG8 interacting motif (AIM) leading to autophagosome formation. The autophagy machinery targets GSNOR1 to lysosome degradation.

Figure 2. *S*-nitrosylation regulates SUMOylation during plant immunity. (A) In the absence of pathogen challenge and the associated nitrosative burst, SUMO conjugating enzyme 1 (SCE1) is not *S*-nitrosylated and consequently SCE1 drives SUMOylation resulting in the negative regulation of plant immunity. (B) Pathogen challenge results in the nitrosative burst leading to higher levels of NO, which results in *S*-nitrosylation of SCE1 at Cys 139, reducing ~~SUMO conjugating enzyme 1 (~~SCE1~~)~~ activity and by extension SUMOylation, contributing to the release of plant immunity suppression.