

REVIEW

Nitric oxide: promoter or suppressor of programmed cell death?

Yiqin Wang¹, Chen Chen^{1,2}, Gary J. Loake³, Chengcai Chu¹ (✉)

¹ National Key Laboratory of Plant Genomics and National Centre for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

² Beijing Forestry University, Beijing 100083, China

³ Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, King's Buildings, Edinburgh EH9 3JR, United Kingdom

✉ Correspondence: ccchu@genetics.ac.cn

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ABSTRACT

Nitric oxide (NO) is a short-lived gaseous free radical that predominantly functions as a messenger and effector molecule. It affects a variety of physiological processes, including programmed cell death (PCD) through cyclic guanosine monophosphate (cGMP)-dependent and -independent pathways. In this field, dominant discoveries are the diverse apoptosis networks in mammalian cells, which involve signals primarily via death receptors (extrinsic pathway) or the mitochondria (intrinsic pathway) that recruit caspases as effector molecules. In plants, PCD shares some similarities with animal cells, but NO is involved in PCD induction via interacting with pathways of phytohormones. NO has both promoting and suppressing effects on cell death, depending on a variety of factors, such as cell type, cellular redox status, and the flux and dose of local NO. In this article, we focus on how NO regulates the apoptotic signal cascade through protein S-nitrosylation and review the recent progress on mechanisms of PCD in both mammalian and plant cells.

KEYWORDS nitric oxide, S-nitrosylation, programmed cell death

PCD IN MAMMALIAN AND PLANT CELLS

Programmed cell death (PCD), or apoptosis in mammalian cells, is a mechanism by which cells activate an intrinsic

suicide program. It is a sophisticatedly regulated cellular process that is essential for organ development and homeostasis (Vaux and Korsmeyer, 1999). PCD is characterized by cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation and selective cleavage of proteins by caspases, which are a family of least 14 mammalian aspartate-specific, cysteine-protease isoenzymes (Hengartner and Bryant, 2000; Meier et al., 2000; Ameisen, 2002). Caspases are the central molecular initiators and executors of apoptosis. They are grouped into 'initiator caspases' and 'effector caspases' by structure and function (Nicholson and Thornberry, 1997; Thornberry and Lazebnik, 1998; Shi, 2002; Nicholson and Thornberry, 2003). Activation of 'initiator caspases' such as caspase-8 and -9 initiates a 'caspase cascade' in which downstream 'effector caspases', such as caspase-3, -6 and -7, are cleaved by upstream initiator caspases to form active heterodimers, which then proteolyze specific protein substrates (Zhou et al., 2005). Caspases also cleave specific aspartate residues in other regulatory proteins, such as B cell lymphoma/leukemia-2 (Bcl-2), Bcl-2 associated x protein (Bax) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) kinase kinase (MEKK). To date, two main apoptosis pathways have been identified in mammalian cells. One is mitochondria-induced cell death, in which cytochrome c is released from mitochondria in the presence of ATP and promotes the assembly of the apoptosome complex, including apoptotic protease-activating factor-1 and procaspase-9. Subsequently, apoptosome-mediated caspase-9 cleavage leads to the activation of effector caspases and results in

cell death. The alternative pathway is mediated by cell surface death receptors, such as tumor necrosis factor receptor (TNFR)-1 or factor associated suicide (Fas), which promote caspase-8 cleavage through adaptor molecules, and lead to apoptosis (Dragovich et al., 1998).

PCD also has an important role in plant growth, development and environmental responses. This process occurs during both the vegetative and reproductive phases of plant development, abiotic stress and interactions with pathogens. In addition to the more obvious forms of PCD, e.g., leaf senescence or hypersensitive response (HR) formation during the plant defense response (Elbaz et al., 2002), alternative programs of PCD include death-terminal differentiation during lysigenous aerenchyma formation and aleurone degradation throughout germination (Jones, 2001). Recent work has highlighted similarities in several aspects of plant and animal PCD formation, such as the formation of apoptotic bodies during HR development. Other morphological similarities, e.g., cytoplasm shrinkage, nuclear condensation and membrane blebbing (Levine et al., 1996; Wang et al., 1996), indicates that the basic molecular machinery underlying this process is well conserved in all eukaryotic organisms (Danial and Korsmeyer, 2004). It appears that collapse of the vacuole may be the universal trigger of plant cell death; however, death during the HR does not result in removal of cell corpses but does release toxins directed against the pathogen. Subsequently, these corpses are crushed by the expanding tissues. Such a "messy" death might be part of the cell's strategy for defense (Jones, 2001). Cathepsins are a family of lysosomal noncaspase cysteine and aspartate proteases involved in mammalian apoptosis. Recent studies on a Cathepsin B (CathB) homologue in *Arabidopsis* and *Nicotiana benthamiana* demonstrated its potential role in promoting HR (Gilroy et al., 2007; McLellan et

al., 2009). In *Arabidopsis*, cathepsin-like proteins also engage autophagic components that function in HR development (Hofius et al., 2009).

NO PRODUCTION IN MAMMALIAN AND PLANT CELLS

In mammalian cells, NO is synthesized as a by-product of the conversion of L-arginine to L-citrulline under the catalysis of a family of enzymes called nitric oxide synthases (NOS) (Fig. 1A). Three NOS isoforms have been identified: one inducible (iNOS, type II), and two constitutive, the neuronal (nNOS, type I) and endothelial (eNOS, type III) enzymes (Marsden et al., 1993; Michel and Feron, 1997). Immune system-related NOS is an inducible and calcium-independent enzyme that produces NO in sufficient quantities to cause deleterious effects to several pathogenic agents (Simmons and Murphy, 1992; Magalhaes et al., 2006). In addition, new isoforms or mitochondrial variants of NOS (mtNOS) have since been described in rat liver (Ghafourifar and Richter, 1997; Giulivi et al., 1998), thymus (Bustamante et al., 2000), and brain (Riobo et al., 2002). mtNOS is bound to mitochondrial cytochrome c oxidase (COX) (Persichini et al., 2005) and to complex I (Franco et al., 2006), thus favoring a steric relationship between the released NO and the control of respiration. NO is critical for numerous biological processes, including PCD, and it functions through both cGMP-dependent and-independent pathways. A key cGMP-independent mechanism is S-nitrosylation (or S-nitrosation), in which protein cysteine thiol reacts with NO in the presence of an electron acceptor to form an S-NO bond. This has emerged as a central, redox-based, post-translational modification (Whiteman et al., 2006).

In plants, three routes to yield NO have been described

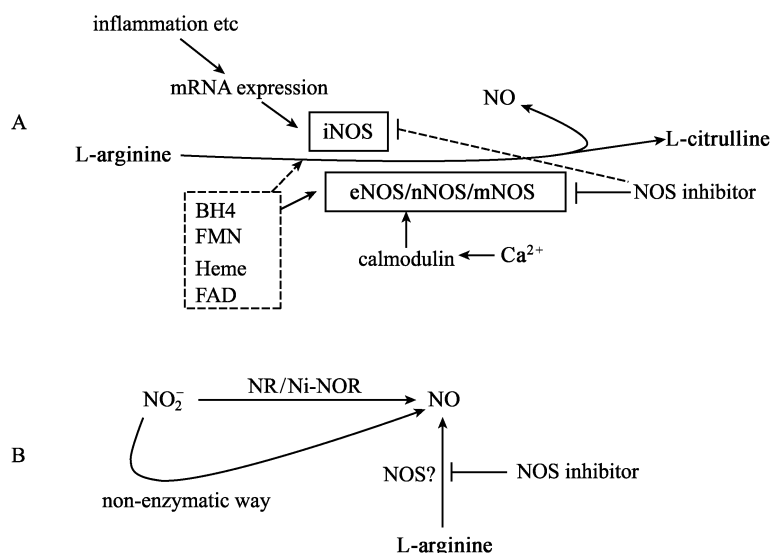


Figure 1. NO production in mammalian (A) and plant (B) cells.

(Fig. 1B): non-enzymatic conversion of nitrite to NO in the apoplast, nitrate reductase (NR)-dependent NO formation, and NOS-like activity that is arginine-dependent (Leitner et al., 2009). Decreased NO synthesis and PCD in the presence of NOS inhibitor suggested the involvement of an NOS-like enzyme in plants, but this enzyme remains enigmatic (Pedroso et al., 2000). One potential candidate for a plant NOS, AtNOA1/RIF1, was subsequently demonstrated not to exhibit NOS activity. Rather, AtNOA1/RIF1 functions as a chloroplast-targeted GTPase, which may be essential for proper ribosome assembly (Moreau et al., 2008).

NO was initially found to play a crucial role in mediating plant defense reactions against bacterial pathogens (Delledonne et al., 1998). In *Arabidopsis*, it was proposed that NO could induce cell death independent of H₂O₂, which is also generated during the defense response, because agents that depleted H₂O₂ production did not affect NO-mediated cell death (Clarke et al., 2000). However, other data suggests that NO and H₂O₂ function in combination to elaborate HR cell death following pathogen recognition (Delledonne et al., 2001).

NO is now well known to influence numerous physiological processes throughout the entire plant life cycle. For example, NO is involved in germination, leaf expansion, lateral root development, flowering, stomatal closure, cell death and defense against biotic and abiotic stresses (Wilson et al., 2008; Leitner et al., 2009). Whereas descriptions of NO-mediated processes are accumulating, the plant signaling pathways governed by NO are still largely unknown. In addition to NO, a series of related molecules, collectively

known as reactive nitrogen species (RNS) are also formed. RNS comprises not only the NO radical (NO[•]) and nitrosonium (NO⁺) ions, but also peroxyxynitrite (ONOO⁻), S-nitrosothiols, higher oxides of nitrogen and dinitrosyl-iron complexes; in short, all NO derivatives can effect NO-dependent modifications (Di Stasi et al., 2002).

CELL DEATH SIGNAL CASCADES REGULATED BY S-NITROSYLATION

Emerging data in animals suggests that S-nitrosylation has a central role in the regulation of cell death. For example, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is S-nitrosylated at its active site, Cys-145, which promotes its association with the E3-ligase, Siah1, resulting in nuclear translocation of the SNO-GAPDH-Siah1 complex (Mannick, 2007). Within the nucleus, SNO-GAPDH stabilizes Siah1 and facilitates the ubiquitination and degradation of nuclear proteins (Hara et al., 2005), while independently, SNO-GAPDH is acetylated and consequently activates the acetyltransferase p300/cAMP-response element-binding protein (CREB) binding protein (CBP), leading to the acetylation/activation of downstream targets, including the tumor suppressor p53 (Sen et al., 2008). Both of these mechanisms promote cell death (Foster et al., 2009). Recently, Sen et al. (2009) reported that a 52 kDa cytosolic protein, GOSPEL, which binds GAPDH in competition with Siah, retains GAPDH in the cytosol and prevents its nuclear translocation. S-nitrosylation of GOSPEL at Cys-47 enhances GAPDH-GOSPEL binding and has neuroprotective action (Fig. 2)

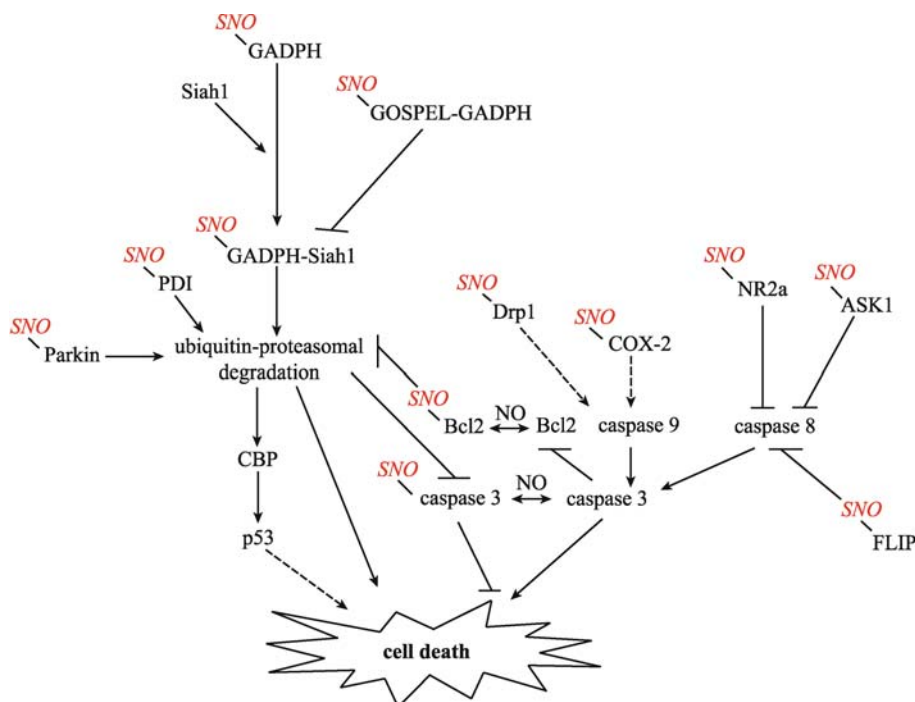


Figure 2. Cell death signal cascades regulated by S-nitrosylation in mammalian cells.

(Sen et al., 2009). How NO selectively reacts with GADPH or GOSPEL in the same cytosolic situation still requires further study, although this may depend on the local concentration of NO.

Under normal physiological conditions, caspase 3 is constitutively S-nitrosylated to prevent it from initiating apoptosis (Tsang and Chung, 2009). When cells receive apoptotic signals, however, caspase 3 is rapidly denitrosylated, and in this form it can cleave a number of key cellular proteins including poly (ADP-ribose) polymerase (PARP), an enzyme important for DNA repair. PARP is cleaved to an 89-kDa fragment, which is often used as an indicator of apoptosis (Lopez and Ferrer, 2000). X-linked inhibitor of apoptosis (XIAP) binds to caspases and inhibits their activation during the execution phase of apoptosis. S-nitrosylation of XIAP does not affect its E3 ligase activity, but instead, it directly compromises its anticaspase-3 and antiapoptotic function (Tsang et al., 2009).

In the mitochondrial induced cell death pathway (Fig. 2), S-nitrosylation of COX-2, the terminal electron acceptor of the electron transport chain, reversibly inhibits mitochondrial respiration and contributes to enhanced oxidant production and PCD of lung cells (Carreras and Poderoso, 2007). The PDZ (postsynaptic density of the *Drosophila* septate junction protein discs-large, and the epithelial tight junction protein ZO-1) domain of nNOS, used as a dominant integrative peptide, blocks nNOS-COX-2 binding and abolishes S-nitrosylation of COX-2 (Tian et al., 2008). NO triggers mitochondrial fragmentation by S-nitrosylating the mitochondrial division protein, dynamin-related protein1 (Drp1). SNO-Drp1 is increased in brains of human Alzheimer's disease patients and may thus contribute to the pathogenesis of neurodegeneration (Cho et al., 2009; Westermann, 2009). Dynamin also proved to be S-nitrosylated at C607 during endocytosis (Wang et al., 2006). On the other hand, NO also has anti-apoptotic function through S-nitrosylation of Bcl2, which inhibits ubiquitin-proteasomal degradation and thus inhibits apoptosis (Azad et al., 2006).

In the extrinsic pathway, binding of membrane ligands like TNF and Fas to membrane receptors triggers the activation of caspases and the induction of apoptosis (Peter and Krammer, 2003). FLICE (FADD-like ICE) inhibitory protein (FLIP) inhibits apoptotic signaling by interfering with the binding of caspase 8 to the adaptor protein at the Fas receptor complex. S-nitrosylation of Cys-254 and-259 on FLIP after Fas stimulation inhibits the ubiquitination and proteasomal degradation of FLIP (Chanvorachote et al., 2005). As a result, FLIP levels are increased and caspase 8 activation and apoptosis are inhibited (Mannick, 2007). Apoptosis signal-regulating kinase 1 (ASK1), another protein that stimulates apoptosis when activated, is also S-nitrosylated on Cys-869, which inhibits its activation by decreasing the binding of ASK1 to its substrate kinases MAPK kinase 3 (MKK3) or MKK6 (Park et al., 2004). Similarly, stimulation of the N-Methyl-D-

Aspartate (NMDA) class of glutamate receptors in neurons leads to increased NOS activation that down regulates NMDA receptor activity and apoptosis via S-nitrosylation of the NR2a regulatory subunit of the NMDA receptor (Choi and Lipton, 2000; Choi et al., 2000; Mannick, 2007). Taken together, S-nitrosylation of different target proteins elaborates diverse and often opposing functions on cell death control. How these modifications are integrated to form a robust system for death control remains a distant goal.

NO FUNCTIONS IN IMMUNITY AND NEURODEGENERATIVE DISEASE

It is becoming evident that NOS activity is associated with human diseases and disorders. Immune cell death is very important for disease development. NO concentration in immune cells plays a significant role in determining thymocyte fate. Recently, it was shown that NO augments the accumulation of activated human T cells principally by inhibiting cytokine deprivation-induced cell death through S-nitrosylation of caspases (Choy and Pober, 2009). The regulation of rat sarcoma/extracellular signal-regulated kinase1 (Ras/ERK) signaling in antigen-stimulated T cells is compartmentalized by the local production of NO. eNOS positively regulates Ras activation during antigen-specific T cell-APC interactions by S-nitrosylation on the Golgi, thus favoring activation-induced T cell death (Ibiza et al., 2008). In human lung carcinoma cells, Caveolin (Cav)-1, a 21–24 kDa structural protein component of plasma membrane microdomains, termed caveolae, has been shown to function in vesicular trafficking, signal transduction, and cancer progression (Galbiati et al., 2001). Cav-1 was rapidly S-nitrosylated by NO after cell detachment and inhibition of this S-nitrosylation by DTT blocked the effect of NO on Cav-1 ubiquitination (Chanvorachote et al., 2009).

S-nitrosylation is also a significant cause of NO-mediated neurotoxicity to neuronal cells, and neural dysfunction leads to neurodegenerative disorders. For instance, during cerebral ischemia, matrix metalloproteinase-9 (MMP-9) colocalizes with nNOS and is S-nitrosylated on a critical cysteine residue, which causes irreversible activation of MMP-9, and as a result, extracellular proteolytic cascades which disrupt the extracellular matrix and contribute to stroke formation are triggered (Gu et al., 2002). NO is thought to play a key role in the pathogenesis of Parkinson's disease (PD) through the impairment of prosurvival proteins, such as parkin, a protective ubiquitin protein ligase that is important for the survival of dopaminergic neurons. Parkin is S-nitrosylated both *in vitro* and *in vivo* in PD patients, indicating that abnormal S-nitrosylation plays an important role in the process of neurodegeneration (Tsang et al., 2009). Furthermore, peroxiredoxin 2 (Prx2) is S-nitrosylated at two critical cysteine residues (Cys-51 and Cys-172), preventing its reaction with peroxides. Increased SNO-Prx2 in human PD

brains was observed, which inhibited both its enzymatic activity and protective function from oxidative stress (Fang et al., 2007). Recent evidence also suggests that NO contributes to degenerative conditions by S-nitrosylating protein disulphide isomerase (PDI), which is located in the ER lumen and prevents neurotoxicity associated with ER stress and protein misfolding (Nakamura and Lipton, 2008). In brains manifesting sporadic Parkinson's or Alzheimer's disease, S-nitrosylation inhibits PDI's enzymatic activity, leading to the accumulation of polyubiquitinated proteins, which activates the unfolded protein response (Uehara et al., 2006). Activation of this stress pathway, may be linked to the development of sporadic neurodegenerative diseases (Uehara, 2007). S-nitrosylation of parkin also eventually leads to the accumulation of unfolded proteins and subsequent neuronal cell death (Nakamura and Lipton, 2009).

SIMILARITIES OF PLANT HR CELL DEATH TO MAMMALIAN PCD

The HR is a form of PCD that is engaged during plant defense against microbial pathogens and is thought to restrict the extent of attempted infection (Mur et al., 2008). HR exhibits some features that are typical of animal cell death, such as chromatin condensation, a requirement for gene expression and activation of a caspase-like cascade (Mur et al., 2008). It was also found that NO inhibits ATP synthesis in plant mitochondria via inhibition of COX activity (Yamasaki et al., 2001) and altered mitochondrial activity stimulates PCD in plant cells. In *Arabidopsis*, NO can function via inhibition of respiration, de-polarisation of the mitochondrial membrane potential and the release of mitochondrial cytochrome c, triggering a signal cascade at the mitochondrial level that accounts for about 20% of total cell death (Zottini et al., 2002). Cyclosporin A, an inhibitor of mitochondrial permeability transition pore (PTP) formation, inhibited NO-induced PCD as well as mitochondrial membrane potential (Saviani et al., 2002). Thus, PTP formation is one of the molecular targets of NO to activate the PCD process in plants. Increased alternative oxidase (AOX) activity has also been observed during pathogen challenge of both *Arabidopsis* and tobacco (Simons et al., 1999). Huang et al. found that exposure to NO induced the expression of AOX. Inhibition of the AOX pathway increased NO sensitivity and cell death, suggesting a 'shunting' of the respiratory pathway via AOX (Huang et al., 2002).

Protein S-nitrosylation is also a significant regulatory component in the plant defense response, which also plays important roles in HR development (Feechan et al., 2005; Romero-Puertas et al., 2007). Caspase like activity during HR induction has been detected in several plants such as tomato, *Arabidopsis* suspension cultures and tobacco (Clarke et al., 2000). Treatment with caspase inhibitors prevented the induction of HR, while no structural homologs of caspases

were found in plant genomes. However, several related proteins, named metacaspases, have been found and proposed to be the functional homologs of mammalian caspases in plant, which are regulators or executors of cell death (Mottram et al., 2003). Based on sequence and structure, nine *Arabidopsis* metacaspase (AtMC) genes have been described. AtMC4 and AtMC9 have been shown to be arginine/lysine-specific cysteine proteases that did not cleave caspase-specific target sites (Vercammen et al., 2004). In the absence of an upstream activator protease, AtMC9 is locked in its inactive proform through S-nitrosylation at Cys-147. Unexpectedly, S-nitrosylation does not affect AtMC9 activity in its processed mature form. A second catalytic cysteine residue (Cys-29 in AtMC9), which is highly conserved in all known metacaspases but is absent in all members of the caspase and paracaspase families, can function as an alternative nucleophile in AtMC9 when Cys-147 is S-nitrosylated. Like mammalian caspases, plant metacaspases can be kept inactive through S-nitrosylation of a critical Cys residue but are insensitive to S-nitrosylation when matured (Delledonne, 2005). S-nitrosylation of *Arabidopsis* cytosolic GAPDH could also play a role in the regulation of cell death, as it causes GAPDH inactivation and potentially induces translocation to the nucleus (Holtgreffe et al., 2008).

NO SIGNALING UNDERLYING PLANT HR INDUCTION

NO and ROS synthesis are routine requirements for plant cells to undergo HR, however, evidence has been presented that either can induce HR independently of the other (Clarke et al., 2000). The emerging evidences also suggest significant cross-talk between NO and ROS (Delledonne et al., 2001), although the molecular details of this interchange remain undefined. Significantly, Delledonne et al. have shown that the NO:superoxide ratio might determine PCD. If superoxide levels are greater than those of NO, then NO reacts with superoxide to form peroxynitrite, which does not result in PCD. Surprisingly, plant cells appear to be relatively tolerant of this particularly reactive molecule. However, if more NO than superoxide occurs, then NO reacts cooperatively with H₂O₂ (arising from dismutation of superoxide) to induce cell death (Delledonne et al., 2001). In tobacco, neither NO nor H₂O₂ alone at low concentrations had any effect on PCD or on the activity of the redox responsive gene that encodes phenylalanine ammonia lyase (PAL). However, treatment with both H₂O₂ and NO together triggered a substantial increase in cell death, with characteristics of PCD, and PAL activity was also induced. In the study of Cd²⁺-induced cell death in *Arabidopsis* cell suspension cultures, NO production precedes ROS accrual following CdCl₂ treatment (De Michele et al., 2009). *Arabidopsis* PrxIIe possesses peroxynitrite reductase activity and therefore might control the

bioavailability of peroxynitrite (ONOO^-) (Romero-Puertas et al., 2007). PrxIIIE is inhibited by S-nitrosylation, causing increased ONOO^- accrual. The increase in this RNS then results in lipidoxidation and tyrosine nitration, which suggests that NO might regulate the effect of its own reactive species through S-nitrosylation of crucial components of the antioxidant defense system (Romero-Puertas et al., 2007). Taken together, these data imply that NO interacts with ROS pathway to affect the cellular redox status which may subsequently mediate the induction of HR cell death.

A number of plant hormones, including auxins, cytokinins, gibberellins, abscisic acid (ABA), jasmonates and ethylene have been implicated in plant cell death control (Tamaoki, 2008). NO routinely exhibits crosstalk with these hormone signaling pathways. For example, in barley aleurone layers, gibberellic acid (GA)-induced PCD was delayed in the presence of NO. This form of PCD is correlated with loss of activity of the antioxidants catalase and superoxide dismutase. However, NO did not inhibit GA-induced alpha-amylase expression and activity, suggesting that NO does not have a general effect on cellular metabolism, but rather acts as a specific endogenous modulator of PCD (Beligni et al., 2002). A novel role for cytokinins in the induction of PCD that required NO but not H_2O_2 has also been proposed (Lamotte et al., 2004). 6-benzyl aminopurine (6-BA) also induces NO production in plants at the level of root tissues, which can be reduced by pretreatment with a NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide (PTIO) and NOS inhibitors.

In ozone-induced HR-like cell death, NO accumulation preceded accumulation of ethylene, salicylic acid (SA) and leaf injury (Rao and Davis, 2001; Ogawa et al., 2005; Tamaoki, 2008). S-nitrosylation of methionine adenytransferase1 (MAT1), which is involved in ethylene biosynthesis, inhibits ethylene production (Lindermayr et al., 2006). Thus, production of this key phytohormone and its associated effects is modulated by NO function. Our recently study on *Arabidopsis* SA binding protein 3 (SABP3) revealed that it is

S-nitrosylated at Cys-280. S-nitrosylation inhibited its carbonic anhydrase activity and SA-binding capacity, which could contribute to a negative feedback loop that modulates the plant defense response (Wang et al., 2009). Non-expression of pathogenesis-related protein 1 (NPR1), a crucial component of disease resistance and SA signal cross-talk, has also recently been found to be regulated by S-nitrosylation of Cys-156, which facilitates its oligomerization and maintains protein homeostasis upon SA induction (Tada et al., 2008). Furthermore, methyl jasmonate (Me-JA) pretreatment of the Cvi-0 accession of *Arabidopsis* decreased ozone-induced SA concentrations and completely abolished ozone-induced cell death. Thus, demonstrating an antagonistic relationship between JA- and SA-signaling pathways in controlling the magnitude of ozone-induced cell death (Tamaoki, 2008). Moreover, allene oxide cyclase (AOC), a JA biosynthetic enzyme, has been shown to be S-nitrosylated during the HR. Hence, NO might also control JA biosynthesis (Romero-Puertas et al., 2008). Collectively, these findings have provided significant insights into our current appreciation of the mechanisms underpinning NO function in plants, revealing that the NO pathway in plant PCD is highly complex and is mediated at least in part by crosstalk with several phytohormone signaling networks.

CONCLUSIONS

PCD, which serves to eliminate unwanted cells, is essential for the maintenance of tissue homeostasis. NO and its related molecules are important messengers that play central roles in this process. This redox molecule regulates PCD through S-nitrosylation of a plethora of target proteins. NO can prevent or induce neuronal apoptosis depending on cellular redox state and its concentration. Different source of NOS may interact with target protein and cause locally high concentration of NO and S-nitrosylation, which can activate a complex network of responses leading to apoptosis via mitochondrial, cell death receptors and GAPDH-Siah1 cascades. The

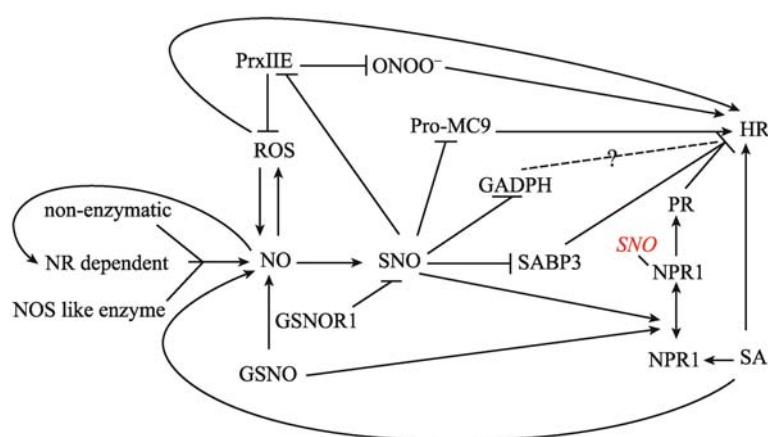


Figure 3. NO signaling regulated by S-nitrosylation in the process of HR.

anti-apoptotic effect of NO can be mediated through a number of mechanisms, such as caspase inactivation, induction of cellular tumor antigen p53 gene expression, upregulation of cellular FLIP, and over-expression of Bcl-2 and Bcl-XL with subsequent inhibition of cytochrome c release from the mitochondria (Iyer et al., 2008). In contrast, hyper-S-nitrosylation of parkin, PDI, Pdx2, XIAP and Drp1 are observed in brains from patients with neurological disorders and S-nitrosylation of these proteins is implicated in stress-induced neuronal cell death (Foster et al., 2009). Thus, there are significant therapeutic opportunities to develop pharmacological agents that modulate NO function, conveying protection against PCD-based neurodegenerative disorders.

In plants, apoptotic characteristics have been observed during development, HR and after abiotic stress, such as exposure to ozone, UV irradiation, chilling and salt stress (Dat et al., 2003). Cell death in plants has also been associated with caspase-like activities. S-nitrosylation of AtMC9, a potential executioner of PCD, is an intriguing example of how NO exerts its physiological functions through S-nitrosylation (Belenghi et al., 2007; Holtgreffe et al., 2008). In addition to metacaspase 9, additional proteins (e.g., NPR1, SABP3, PrxIIIE, GADPH and MAT1) that might function in PCD, have been shown to be regulated by S-nitrosylation (Lindermayr et al., 2006; Romero-Puertas et al., 2007; Holtgreffe et al., 2008; Tada et al., 2008; Wang et al., 2009). These data demonstrate the important regulatory role of NO in plant PCD, which is just beginning to emerge. The current data implies that cell death control in plants may be underpinned by mechanisms distinct from those found in animals. Uncovering this molecular machinery may therefore reveal novel strategies for the regulation of PCD.

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ABBREVIATIONS

ABA, abscisic acid; AOC, allene oxide cyclase; AOX, alternative oxidase; ASK1, apoptosis signal-regulating kinase 1; 6-BA, 6-benzyl aminopurine; Bax bcl-2 associated x protein; Bcl-2, B cell lymphoma/leukemia-2; Cav-1, Caveolin-1; CBP, CREB binding protein; cGMP, cyclic guanosine monophosphate; COX, cytochrome c oxidase; CREB, acetyltransferase p300/cAMP-response element-binding protein; Drp1, dynamin-related protein1; FLIP, FLICE inhibitory protein; HR, hypersensitive response; MAPK, mitogen-activated protein kinase; MAT1, methionine adenosyltransferase 1; MC, metacaspase; MEKK, MAPK/ERK kinase kinase; MKK3, mitogen activated protein kinase kinase-3; MMP-9, matrix metalloproteinase-9; NO, nitric oxide; NOS, nitric oxide synthases; NPR1, non-expressor of pathogenesis-related protein 1; NR, nitrate reductase; PAL, phenylalanine ammonia-lyase; PARP, poly(ADP-ribose) polymerase; PCD,

programmed cell death; PD, Parkinson's disease; PDI, protein disulfide isomerase; PDZ, postsynaptic density of the *Drosophila* septate junction protein discs-large, and the epithelial tight junction protein ZO-1; PrxIIIE, peroxiredoxin II E; PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; PTP, permeability transition pore; Ras/ERK, reliability, availability and serviceability/extracellular signal-regulated kinases; RNS, reactive nitrogen species; SA, salicylic acid; SABP3, SA binding protein 3; TNFR, tumor necrosis factor receptor; XIAP, X-linked inhibitors of apoptosis.

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