Protein S-Nitrosylation in Plants: Photorespiratory Metabolism and NO Signaling

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Protein *S*-Nitrosylation in Plants: Photorespiratory Metabolism and NO Signaling

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The highly diffusible free radical nitric oxide (NO) has emerged as a key signaling molecule in bacteria, plants, and animals. There are several mechanisms through which NO is produced in plants, and once produced, NO readily reacts with various targets, such as thiols and the metallic centers of proteins. During the past few years, *S*-nitrosylation, the covalent and reversible binding of NO to the thiols of reduced reactive cysteine residues, has emerged as an important posttranslational modification. *S*-nitrosylation is thought to account for much of the widespread influence of NO on cellular signaling through redox-based biochemical regulation of signaling components. Here, I highlight the emerging roles of *S*-nitrosylation in plants with particular emphasis on the role of *S*-nitrosylation in mitochondria during the defense response.

Over the past few years, nitric oxide (NO) has emerged as a versatile signaling molecule. In virtually all organisms, this small, lipophilic free radical is synthesized by various pathways (*1*) and has a very short biological half life (5 to 15 s). It can readily diffuse within a cell or between neighboring cells to react with various intracellular and extracellular targets such as thiols or the catalytic metal centers of proteins. Important NO-dependent protein modifications in biological systems include covalent modifications of tyrosine (tyrosine 3-nitration) or cysteine (*S*-nitrosylation) residues and binding of NO to transition metals (metal nitrosylation). To date, the best-characterized covalent modification is cysteine *S*-nitrosylation, which occurs on a time scale of seconds to minutes and plays a role in regulating various cellular signaling processes. There are several enzymes, such as *S*-nitrosoglutathione reductase (GSNOR) and some thioredoxins, that can degrade *S*-nitrosothiols (*2*, *3*) to increase their turnover rates. Proteomic analysis has been instrumental in identifying several plant proteins that are nitrosylated in cultured cells and whole leaves after treatment with NO donors or NO gas (*4*), and 16 proteins were found to be nitrosylated during the hypersensitive response (HR), a form of pathogen-elicited programmed cell death, in *Arabidopsis thaliana* (*5*). Despite extensive research, the functional roles of only a few nitrosylated proteins have been elucidated to date, and much remains to be resolved; therefore, *S*-nitrosylation is an emerging topic in plant NO research.

One recent finding is that *S*-nitrosylation regulates oligomerization and localization of nonexpressor of pathogenesis-related genes 1 (NPR1), a redox-regulated protein that plays a major role in the defense response (Fig. 1) (*6*). In uninfected plants, NPR1 resides in the cytosol as an oligomer in which monomers are linked by disulfide bonds. Upon pathogen challenge, salicylic acid (SA) production increases, leading to the thioredoxin-catalyzed dissociation of NPR1 oligomers into monomers, which can translocate into the nucleus. *S*-nitrosylation of NPR1 stimulates its oligomerization (*7*). More recently, Lindermayr *et al*. reported that *S*-nitrosylation of purified recombinant NPR1 and TGACG motif binding factor 1 (TGA1), a transcription factor with which NPR1 associates, promoted translocation of NPR1 into the nucleus and enhanced TGA1 binding to the activation sequence–1 element present in promoter regions of several defense-related genes (*8*). In this study, *S*-nitrosylation–mediated oligomerization was not shown to inhibit NPR1 translocation (*8*), so the mechanism by which the dynamics of NPR1 oligomer-monomer interconversion regulate nuclear translocation is unclear. It is also not clear whether the nitrosylated or denitrosylated form of NPR1 translocates into the nucleus. What is clear is that NPR1 nuclear translocation occurs only in the presence of NO (*7*, *8*). These observations are compatible with previous studies indicating that the global increase in global *S*-nitrosothiols in *GSNOR* mutants compromises SA signaling and disease resistance, whereas overexpression of *GSNOR* leads to increased SA signaling and disease resistance (*9*). NO may further influence redox events through nitrosylation of the HR regulator peroxiredoxin IIE (PrxIIE), which leads to inhibition of its activity and enhanced protein tyrosine nitration (*1*).

Mitochondria are also important in NO biology. In addition to being involved in adenosine 5′-triphosphate production and regulation of nuclear gene expression through retrograde signaling, mitochondria generate NO through arginine- and nitrite-dependent pathways. *S*-nitrosylation may play a specific role in mitochondria due to the abundance of transition metals and thiols in this organelle. Palmieri *et al*. recently identified 11 *Arabidopsis* mitochondrial proteins as targets for *S*-nitrosylation (*10*), and they examined mitochondrial NO formation in *Arabidopsis* cell cultures that had been treated with the pathogen-derived factor harpin (found in *Erwinia*, *Pseudomonas*, and *Xanthomonas*), which induces NO production and cell death. Harpin did elicit NO production in mitochondria, as indicated by colocalization of the NO-specific fluorescent dye diaminofluorescein and the mitochondrial marker MitoTracker Red in cultured *Arabidopsis* cells. *S*-nitrosoglutathione (GSNO) treatment of partially purified mitochondria caused nitrosylation of 11 proteins, three of which were subunits of the glycine decarboxylase complex (GDC), a key enzyme of the photorespiratory C_2 cycle: glycine dehydrogenase subunit P2, glycine decarboxylase subunit H1, and glycine dehydrogenase subunit P1. Palmieri *et al*. also showed that GDC activity decreased by up to 70% when isolated mitochondria were incubated with GSNO, whereas the activity increased by up to 67% after treatment with dithiothreitol or glutathione, which are reducing agents that break disulfide bonds between cysteine residues. GSNO suppressed the activity of partially purified GDC by inhibiting both the P and H subunits.

One major physiological effect of NO-mediated regulation of GDC is on photorespiration, an auxiliary metabolic process that enables plants to thrive in the presence of oxygen. Excessive excitation energy, which is light in excess of the amount that the plant needs to accomplish photosynthesis, leads to an overreduction of the electron-transport chain of the photosynthetic light reactions and a concomitant increase in oxygen, which produces reactive oxygen species (ROS). Excess ROS are detrimental to photosyn-

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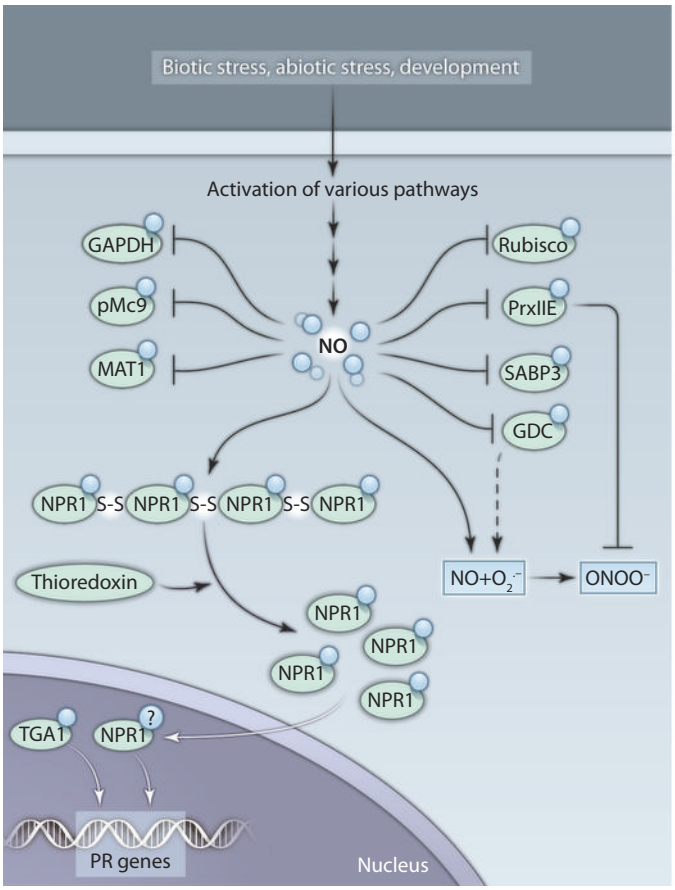

Fig. 1. Many potential targets for *S*-nitrosylation have been identified in plants. Under normal conditions, basal levels of NO cause *S*-nitrosylation (blue circles) of NPR1 to keep NPR1 in its oligomeric form. Upon induction of the defense response by pathogens, NO is produced and thioredoxins promote monomerization of NPR1. NPR1 monomers translocate to the nucleus, but whether the nitrosylated or denitrosylated form translocates is unclear. Nuclear NPR1 interacts with the nitrosylated form of the transcription factor TGA1 to induce transcription of pathogenesis-related genes. NO may promote cell death in the pathogen response in multiple ways. First, nitrosylation inhibits GDC, and the resulting increase in ROS (shown as $O_2^{\cdot-}$ in the diagram) leads to an increase in peroxynitrite formation and subsequent cell death. *S*-nitrosylation of PrxIIE leads to inhibition of its peroxynitrite ($ONOO^-$) reductase activity and, thus, also promotes cell death. Inhibition of salicylic acid binding protein 3 (SABP3) by nitrosylation contributes to the negative feedback loop for plant defense. RuBisCO activity can be inhibited by nitrosylation, and this can decrease photosynthesis. *S*-nitrosylation also inhibits glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the ethylene biosynthetic enzyme methionine adenosyltransferase 1 (MAT1), and the cell-death effector prometacaspase 9 (pMC9).

thesis because they cause lipid peroxidation, modification of thylakoid stromal proteins, and inactivation of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) (*11*). During photorespiration, the initial enzyme of the photosynthetic Calvin-Benson cycle, RuBisCO, adds oxygen to the sugar ribulose 1,5-bisphosphate instead of carbon dioxide, its substrate during normal photosynthesis. This reaction generates the noxious compound glycolate 2-phosphate, which is hydrolyzed to glycolate by a chloroplastidial phosphatase. Two peroxisomal enzymes then convert glycolate into glycine, after which glycine enters mitochondria, where glycine decarboxylase and serine hydroxymethyltransferase convert two molecules of glycine into one molecule of serine. During this process, one molecule of CO_2 is released (hence the term "photorespiration"), and the reduced form of nicotinamide adenine dinucleotide (NADH) is generated. Back in the peroxisomes, serine is converted into glycerate, and this glycerate is further processed in the chloroplasts to yield glycerate 3-phosphate, which reenters the Calvin-Benson cycle (*12*). Given the large amounts of hydrogen peroxide produced during glycolate oxidation and multiple connections to energy and amino acid metabolism, malfunction of the photorespiratory cycle (for example, by inhibition of GDC) can substantially increase ROS levels and affect redox signaling.

Photorespiration also affects some plant-pathogen interactions; thus, it was interesting that the Palmieri *et al*. study showed that a 4-hour incubation of leaf slices with harpin led to a 60% decrease in GDC activity and an increase in the glycine-to-serine ratio, indicating reduced photorespiratory capacity (*10*). However, it was unclear whether the effect of harpin-induced NO on GDC activity was direct or indirect. GSNO treatment inhibits complex I, a component of the mitochondrial electron-transport chain, and this increases ROS in mitochondria, which could later affect chloroplastic ROS through reduced photorespiratory capacity. Therefore, it is possible that the change in photorespiratory capacity after harpin treatment was a secondary consequence of increasing mitochondrial ROS rather than a direct effect of inhibiting GDC. To address this issue, Palmieri *et al*. incubated purified mitochondria with the complex I inhibitor rotenone but found no change in GDC activity, suggesting that NO, but not ROS, was responsible for inhibiting GDC activity. Further emphasizing the central role of GDC inhibition in ROS generation, treating cultured cells with the potent GDC inhibitor aminoacetonitrile (AAN) led to an increase in ROS production. Increased staining with Evans blue, a dye that penetrates only damaged or dying cells, confirmed that cell death increased after treatment with AAN or harpin. These results suggest that *S*-nitrosylation of GDC promotes cell death in the HR through increased ROS production.

In this respect, programmed cell death (PCD) in plants appears to be similar to the well-characterized mammalian apoptotic form of PCD. In mammals, NO promotes apoptosis by reversibly binding to mitochondrial cytochrome c oxidase to increase superoxide formation. The superoxide, in turn, reacts with peroxynitrite, which can cause release of cytochrome c from mitochondria, a key trigger of mammalian apoptosis. Although cytochrome c release has been noted during plant PCD, its role remains obscure (*13*). Plants have an alternative to cytochrome c in alternative oxidase (AOX), which can accept electrons directly from the ubiquinone pool, thereby preventing excessive reduction of ubiquinone and ROS production. Treatment with NO donors increases AOX expression and activity (*14*), suggesting a dichotomy wherein NO could increase chloroplastic ROS by reducing mitochondrial ROS during the HR, for example. It could be that GDC inhibition by NO-mediated nitrosylation counteracts the antioxidant effects of AOX. This differential control may reflect the relative concentration of NO in mitochondria, and specific concentrations of ROS and NO appear to be important in the progression or suppression of cell death in plants (*15*).

The Palmieri *et al*. study has opened new horizons and posed many questions

regarding the role of mitochondria in plant PCD. Clearly, more work is needed to determine how GDC inhibition increases ROS. In particular, photorespiratory oxidation of glycine to serine by serine hydroxymethyl transferases in the mitochondrial matrix needs to be examined, because this is coupled to NADH generation. Therefore, GDC inhibition could limit the supply of NADH to the electron-transport chain, resulting in a change in cellular redox status. Additional studies are needed to relate GDC effects to NO generation in mitochondria and to clarify the effect of *S*-nitrosylation on antioxidant enzymes. It would also be interesting to determine the effect of peroxynitrite and NO on GDC activity. This study has unequivocally provided evidence for the importance of mitochondrial *S*-nitrosylation in the plant defense response.

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