Protein *S*-Nitrosylation: Potential Targets and Roles in Signal Transduction

Posttranslational modification of proteins potentially alters physical and chemical properties of the protein and, as a result, the function. Rapidly reversible modifications, such as phosphorylation, acylation, glycosylation, ubiquitination, and *S*-nitrosylation, can play an important role in the regulation of dynamic processes such as pathogen response and metabolism. The knowledge of consensus sequences surrounding these sites has allowed database searches for proteins that can potentially undergo posttranslational modification. However, these possible targets need to be verified at the biochemical, molecular, and physiological levels. This month's *High Impact*, ''Proteomic Identification of *S*-Nitrosylated Proteins in Arabidopsis'' by Lindermayr et al., starts to address this in regards to protein *S*-nitrosylation. This article appeared in our March 2005 issue and as of April 2007 had 37 citations (Thompson ISI Web of Science, http://www.isinet.com).

BACKGROUND

Nitric oxide (NO), an emission from combustion processes such as fossil fuel-burning power plants and automobiles, is a reactive gas that is considered toxic when found in the environment. In plants and animals, however, it is a diffusible molecular messenger that plays an important role in signaling processes. The role of NO was first identified in plants by its involvement in regulation of growth and hormone signaling (Guo et al., 2003) and later as a messenger in plant defense against microbial pathogens.

In animals, NO is synthesized by a conserved family of NO synthases (NOS), located in the cytosol or membrane bound. Animal NOS convert L-Arg to NO and L-citrulline. No ortholog of animal NOS family members has been identified in plants to date. One gene in Arabidopsis (*Arabidopsis thaliana*), *AtNOA1* (formally *AtNOS1*), has been identified to be involved in NO synthesis or accumulation (Guo et al., 2003). However, this protein bears no sequence similarity to mammalian NOS (Guo et al., 2003), nor does it have the Arg-dependent (NOS) activity in vitro exhibited by animal NOS (Crawford et al., 2006; Zemojtel et al., 2006). *AtNOA1* appears to be activated by hormones, and knockouts of this gene have reduced growth and fertility and increased susceptibility to microbial pathogens (Guo et al., 2003; Zeidler et al., 2004). Another source of NO in plants is through the enzymatic activity of nitrate reductase. Nitrate reductase transcript and protein levels increase in response to a fungal pathogen or its elicitor in potato (*Solanum tuberosum*) tubers, suggesting a role for nitrate reductase in the synthesis of NO during the defense response (for review, see Delledonne, 2005).

Within the plant, NO can react with sulfhydryl groups on proteins, yielding *S*-nitrosothiols, which lead to a change in protein function or activity. *S*-Nitrosylation of proteins is rapidly reversible, making it an attractive candidate for involvement in signal transduction. NO can also react with transition metals to produce metal nitrosyls. It is uncertain which of these two processes is the more dominant modification in plants or even the targets of either. In animal systems, NO binds to soluble guanylate cyclase, activating the enzyme and increasing levels of the second messenger cGMP. There is evidence in plants that NO can transiently increase levels of cGMP (for review, see Wendehenne et al., 2004), although the process for this is not known. Database searches have identified some of the potential targets of NO, but few have been experimentally confirmed.

WHAT WAS SHOWN

The study by Lindermayr et al. (2005) partly addresses the following question: What are the targets of NO (specifically, which proteins are *S*-nitrosylated in Arabidopsis)? A proteomics approach coupling nano liquid chromatography and tandem mass spectrometry with biotin switch detection and purification identified 63 modified proteins from cell culture extracts treated with the NO donor *S*-nitrosoglutathione (GSNO) and 52 modified proteins from leaves exposed to NO gas. Biotin switch detection is highly specific for *S*-nitrosylated proteins and has been used successfully in animals systems (Jaffrey et al., 2001). *S*-Nitrosylated proteins are labeled on *S*-nitrosylated Cys residues with a biotin moiety enabling detection with anti-biotin antibodies (Jaffrey and Snyder, 2001).

The 63 proteins identified from GSNO-treated cell culture extracts included proteins involved in stress-related responses, metabolism, signaling, and regulation; redox-regulated proteins; and cytoskeleton-associated proteins. Several of the stress-related proteins are homologous to those demonstrated to undergo *S*-nitrosylation in animal systems and thus may be important under oxidative and nitrosative stress conditions. Among the metabolic enzymes identified as possible targets of *S*-nitrosylation were five glycolysis enzymes, including GAPDH, which contains a Cys residue in the reactive site known to be inhibited by NO. Sulfur metabolism enzymes were also identified, including *S*-adenosylmethione that is inhibited by NO in rats. *S*-Adenosylmethione is a substrate for the biosynthesis

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of ethylene, leading the authors to hypothesize that the *S*-nitrosylation of *S*-adenosylmethione and other members of the methylation cycle might mediate the cross talk between NO signaling and ethylene, whose synthesis is regulated by NO. Recently, it was reported that the methionine adenosyltransferase isoforms in Arabidopsis undergo differential inhibition by NO, lending further support to a possible regulatory role for *S*-nitrosylation in the negative regulation of ethylene biosynthesis (Lindermayr et al., 2006).

Many of the chloroplast proteins identified in this study, specifically those of the Calvin-Benson cycle, are regulated in a redox-dependent manner. PSII proteins were also identified as targets of *S*-nitrosylation; indeed, inhibition of reversible PSII photophosphorylation by NO has been demonstrated previously (Takahashi and Yamasaki, 2002). The confirmation that *S*-nitrosylation does occur in the identified proteins is an important next step.

THE IMPACT

Evidence is mounting for the involvement of NO in stress conditions. Using olives (*Olea europaea*) subjected to NaCl stress, Valderrama et al. (2007) found an increase in NO and NO-derived products, including *S*-nitrosylated proteins, indicating a role for these compounds during NaCl stress. Stress-related proteins were also among the proteins Lindermayr et al. (2005) found in their proteomic study to be *S*-nitrosylated. In general, Valderrama et al. (2007) observed an increase in NO and the NO-derived *S*-nitrosothiols, GSNO and 3-nitrotyrosine, in the vasculature as well as all cell types of olive due to NaCl stress, whereas in control plants NO was limited to the vascular tissue, similar to what has been observed in other studies. The increased presence in the vasculature was interpreted as a way to redistribute NO-derived molecules.

GSNO can act as an NO reservoir as well as an NO donor and can be reduced by GSNO reductase (GSNOR). GSNOR controls not only the cellular levels of GSNO but also the levels of *S*-nitrosylated proteins. In animal systems, GSNOR enhances resistance to nitrosative stress, while in plants it has a role in disease resistance (Feechan et al., 2005; Rustérucciet al., 2007). This role in disease resistance has been demonstrated in Arabidopsis by T-DNA insertion in *AtGSNOR* (Feechan et al., 2005) and antisense technology (Rustérucci et al., 2007). Although the results from these studies are contradictory—the T-DNA lines had decreased pathogen resistance while the antisense plants had increased resistance to pathogens—both studies highlight the importance of GSNOR and NO in disease resistance. This dissimilarity in results could possibly arise from differences in plant ages. Other studies have reported that NO can reduce pathogen-induced cell death (for review, see Mur et al., 2006).

Signaling by reactive oxygen species, specifically hydrogen peroxide (H_2O_2), is known be instrumental in several plant processes, including regulation of programmed cell death. In the hypersensitive response, there is both an oxidative and a nitrosative burst prior to activation of the signal cascade that eventually activates the transcription of defensive genes (for review, see Zaninotto et al., 2006). It is this balanced coproduction of reactive oxygen species and NO that ultimately leads to the cell death observed during hypersensitive response, but how the program is triggered is unknown (for review, see Zaninotto et al., 2006). Also unknown are the proteins that interact directly with H_2O_2. Since H_2O_2 and NO are known to operate together in signaling cascades, the identification of proteins that interact with both H_2O_2 and NO could allow the discovery of the point of overlap between the two pathways. A recent study by Hancock et al. (2005) identified proteins that interact with H_2O_2. The key protein identified is GAPDH, which was also shown to undergo *S*-nitrosylation by Lindermayr et al. (2005) and could possibly be the link between these two important pathways.

CONCLUSION

S-Nitrosylation of proteins is proving to be an important component of plant cell regulation, and the very transitory nature of this posttranslational modification lends itself well to this role. Now that some proteins that are potentially regulated in this manner have been identified, the next step is to determine if indeed they are modified by NO and the role of this posttranslational modification in plants.

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