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Preview

Modulating the Modulator: Regulation of Protein Methylation by Nitric Oxide

Lucas Frungillo[1] and Steven H. Spoel[1,*]
[1]Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3BF, UK
*Correspondence: steven.spoel@ed.ac.uk
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Protein methylation is an important modulator of signal transduction pathways, but methyltransferases themselves may also be modulated. Hu et al. (2017) demonstrate in this issue of *Molecular Cell* that *S*-nitrosylation selectively modulates enzymatic activity of a protein arginine methyltransferase vital to abiotic stress tolerance.

Eukaryotic cell developmental and stress-response programs involve a variety of reactive oxygen and nitrogen species, including the free radical nitric oxide (NO), that act as short- and long-distance signaling molecules. One of the main signal transduction mechanisms of NO is derived from its ability to reversibly bind cysteine (Cys) thiols to form post-translational, redox-sensitive *S*-nitrosothiol (SNO) modifications. *S*-nitrosation, often referred to as *S*-nitrosylation, has profound effects on the behavior of proteins by regulating their activity, localization, structure, and interaction with biomolecules. Consequently, NO has been found to regulate diverse cell signaling processes including gene expression. More recently, it has emerged that *S*-nitrosylation shows complex interplay with several other post-translational modifications, thereby expanding the large repertoire of cell signaling pathways it regulates (Skelly et al., 2016). In this issue of *Molecular Cell*, Hu et al. (2017) extend our knowledge on the pervasiveness of NO in post-translational signaling by demonstrating that *S*-nitrosylation of protein arginine N-methyltransferase 5 (PRMT5) in plants promotes its methyltransferase activity, which enables methylation-dependent pre-RNA splicing associated with salt stress tolerance. These findings add an important new layer of complexity to NO signaling, as they indicate that NO not only reprograms gene transcription but also controls the post-transcriptional production of functional mRNA transcripts and perhaps can further diversify the proteome through alternative splicing.

Quantitative reactivity profiling of functional cysteines has shown that relatively few cysteines in the eukaryotic proteome are hyper-reactive and that their position often correlates with enzymatic active sites (Weerapana et al., 2010). Indeed, enhanced nucleophilicity of cysteines in or near the active site is an indispensable feature of many catalytic enzymes. Consequently, *S*-nitrosylation of enzymes is often associated with inhibition of enzymatic activity by directly blocking active site cysteines, by blocking substrate access, or by causing secondary effects such as conformational change or cofactor exclusion. Compared to those that are inhibited, only a handful of enzymes are stimulated by *S*-nitrosylation. Hu et al. (2017) now add to this short list by showing that *S*-nitrosylation of Cys125 of PRMT5 promotes its methyltransferase activity. Although the exact mechanism remains unclear, the N terminus of PRMT5 contains a TIM barrel domain that incorporates Cys125. Crystal structure and biochemical analyses of prototypic *Caenorhabditis elegans* PRMT5 suggest that the TIM barrel aids in the homodimerization of PRMT5 (Sun et al., 2011). Although Cys125 is not conserved in *C. elegans* (Figure 1A), it is plausible that in *Arabidopsis thaliana* PRMT5 *S*-nitrosylation affects TIM barrel homodimerization and, consequently, activity.

Alternatively, *S*-nitrosylation of Cys125 may play a role in establishing substrate selectivity of PRMT5. Hu et al. (2017) show that severe developmental defects in *prmt5* mutant plants are fully rescued by expression of a mutant PRMT5(C125S) transgene, whereas hypersensitivity to salt stress was maintained. Because salt stress induces an NO burst and is associated with *S*-nitrosylation of PRMT5, modification of Cys125 may specifically focus PRMT5 activity during periods of environmental stress as opposed to normal development. So how could PRMT5 *S*-nitrosylation establish substrate selectivity during stress responses? Human PRMT5 is part of a methylosome complex, and its TIM barrel region interacts with pICln, a highly conserved protein that associates with spliceosomal Sm proteins and mediates assembly of the RNA splicing machinery. Binding of pICln focuses PRMT5 activity on Sm proteins and away from other substrates such as histones (Pesiridis et al., 2009). Interestingly, the adaptor protein RioK1 competes with pICln for binding to PRMT5 and recruits the RNA-binding protein nucleolin for methylation (Guderian et al., 2011). Although human PRMT5 does not contain a homologous Cys residue (Figure 1A), it is plausible that *S*-nitrosylation of Cys125 of the TIM barrel domain in *A. thaliana* PRMT5 enables adaptor protein switching to establish substrate specificity.

Although highly conserved among plants, Cys125 does not appear to be conserved in canonical human PRMTs (unpublished data). This observation raises intriguing questions about the selective forces that drove evolution of this potentially unique post-translational control mechanism for plant PRMT5. Nevertheless, human PRMTs contain several other potentially reactive Cys residues (Figure 1A). Given their importance in disease development and progression (Yang and Bedford, 2013), investigation of redox-mediated control of human PRMT activity may prove a fruitful

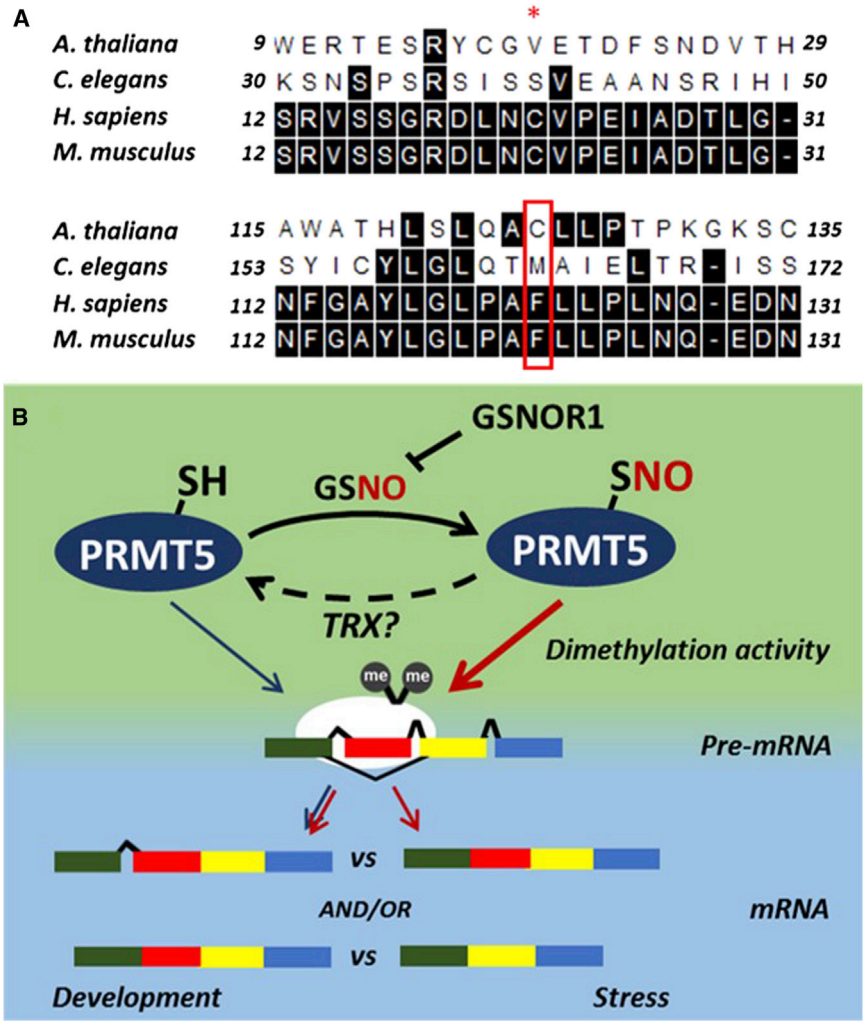

Figure 1. Model for the Role of *S*-Nitrosylation in Regulating PRMT5 Activity and Associated Post-transcriptional Effects during Plant Development and Environmental Stress

(A) Alignment of PRMT5 protein sequences from selected organisms. Shaded letters indicate sequence identity, and numbers refer to the position of the amino acid residues in each protein. In human and mouse PRMT5, Cys22 is predicted (GPS-SNO software) to be targeted by *S*-nitrosylation (red asterisk). Additionally, while conserved among plant species (Hu et al., 2017), the site of *S*-nitrosylation in plant PRMT5 (Cys125) is not conserved among animals (red box). Both Cys22 and Cys125 residues are located within the regulatory TIM barrel region of PRMT5.

(B) Schematic representation for the multi-layered impact of *S*-nitrosylation on the proteome. At the protein level (green shading), GSNO mediates *S*-nitrosylation of PRMT5 (PRMT5-SNO). PRMT5 *S*-nitrosylation is indirectly controlled by GSNO reductase (GSNOR1) activity and may also be directly reversed by the activity of thioredoxins (TRX). *S*-nitrosylation of PRMT5 stimulates its dimethylation (-me) activity on spliceosome components (white oval), resulting in regulation of the proteome through post-transcriptional mechanisms (blue shading). Two—not mutually exclusive—mechanisms of post-transcriptional regulation are proposed. Under conditions of stress, PRMT5-SNO promotes pre-mRNA processing to yield functional mRNA transcripts in response to environmental cues. Alternatively, PRMT5 and PRMT5-SNO are proposed to generate different splice variants during cell development and environmental stress, leading to diversification of the proteome.

strategy to build on the limited knowledge of its post-translational control and to provide novel avenues for therapeutic strategies.

Because stress-induced *S*-nitrosylation of PRMT5 stimulates its activity and may alter substrate specificity, mechanisms must be in place to fine tune the occurrence and duration of this modification. Hu et al. (2017) show here that PRMT5 *S*-nitrosylation is strongly enhanced in plants that carry mutations in *S*-nitosoglutathione reductase (GSNOR1). GSNOR1 indirectly controls cellular protein-SNO levels through its reducing activity toward *S*-nitosoglutathione, a naturally occurring NO donor capable of trans-nitrosylating proteins (Feechan et al., 2005). Additionally, cellular protein-SNO levels are controlled by the thioredoxin (TRX) family of oxidoreductases, which directly denitrosylate proteins through hetero- or homolytic cleavage of the S-NO bond. Plant cells contain numerous TRX proteins in all organelles, yet a recent study suggests that specific TRX proteins are induced by environmental stress and, accordingly, selectively target specific substrate repertoires (Kneeshaw et al., 2014). In addition to indirect regulation by GSNOR1, *S*-nitrosylation of PRMT5 may also be directly controlled by specific, stress-inducible TRX proteins (Figure 1B). If *S*-nitrosylation of other PRMT5 Cys residues (Cys260 and Cys425) (Hu et al., 2017) is found to be biologically relevant, coordination of the enzymatic denitrosylation network may provide control points at each residue and could represent an important step forward in understanding specificity in cellular SNO signaling.

Arguably the most exciting finding presented by Hu et al. (2017) is that, through its effect on PRMT5 methyltransferase activity, NO controls pre-mRNA processing. It is demonstrated that mutation of Cys125 resulted in aberrant splicing of pre-mRNA of a salt stress-related gene but did not affect the splicing of a developmental gene, suggesting that NO modulates pre-mRNA splicing only during conditions of stress. RNA-seq analyses have demonstrated that mutation of *PRMT5* causes splicing defects in hundreds of genes, leading to a reduction in functional transcripts and associated protein levels (Deng et al., 2010). Moreover, PRMT5 has been reported to be involved in regulation of alternative splicing. Thus, NO may uniquely diversify the stress-induced proteome through PRMT5-SNO-mediated regulation of functional transcripts and formation of new splice variants (Figure 1B). In addition to its post-translational regulatory role, these findings highlight exciting new post-transcriptional mechanisms by which NO

cross talks with other modifications to impact the proteome.

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