Nitric oxide (NO)-mediated signaling has been discovered in life forms ranging from bacteria to plants to humans. Because of its intrinsic reactivity, this small and freely diffusible molecule has several mechanisms of action. Direct binding of NO to and activation of soluble guanylate cyclase was discovered first and has been studied most. However, another important NO-mediated signaling, which occurs through modification of protein thiol residues, always draws attention. Recent advances in detection methods of S-nitrosothiols, such as the biotin switch or SNO-RAC isolation coupled with mass spectrometry-assisted target protein identification, led to the discovery of proteins prone to this modification. Protein S-nitrosation (aka S-nitrosylation) is an indirect reaction that can proceed by multiple pathways. In most cases, NO must first be oxidized to NO+, often by oxygen or by a transition metal, in order to modify protein cysteine residues. As both of these reactions can proceed nonenzymatically and S-nitrosylation is ubiquitous, it was originally assumed that the proximity to a NO+ source solely defined the reaction specificity (Derakhshan et al., 2007). However, the propensity of nitroso groups to transfer from one thiol to another (trans-nitrosylation) opposed this point of view (Marino and Gladyshev, 2010). Two articles published in this issue of Molecular Cell further developed our knowledge of this type of NO signaling. Seth et al. (2018) demonstrated that S-nitrosylation is an enzymatic reaction leading to specific protein modifications, whereas Wolhuter et al. (2018) examined the stability and functionality of S-nitrosylation.

Building on their previous studies of proteins S-nitrosylation in E. coli as a model organism, Seth et al. (2018) demonstrated that, at least under anaerobic conditions, when NO oxidation by oxygen is impossible, the hybrid cluster protein (Hcp) is responsible for most of cellular protein S-nitrosylation. Hcp is found in a large multiprotein complex together with the nitrate reductase and trans-nitrosylases. Hcp catalyzes auto-S-nitrosylation of a coordinating cysteine residue via an Fe-mediated redox reaction, indicating that iron-sulfur cluster proteins, found in all the phyla, can act as specific nitrosylases. Other protein components of this complex facilitate trans-nitrosylation and thus specific propagation of nitrososignaling. These results demonstrate that in anaerobically grown E. coli S-nitrosylation is essentially an enzymatic reaction specific to a group of target proteins. S-nitrosylation of some of these targets is required to obtain a beneficial phenotype, namely “swimming” motility. Whereas the exact molecular mechanism of NO-dependent “swimming” lies outside of the scope of this report, the results presented...
therein clearly indicate that NO-mediated signaling is required for increased mobility under anaerobic conditions. Moreover, Hcp-mediated S-nitrosylation primes the cells to become resistant to subsequent nitrosative stress and, together with NO reductase, promotes their growth under this condition.

Wolhuter et al. (2018) performed extensive work to challenge the common assumption that $[^{\text{3}}\text{C}]SN^{\text{O}}$ is a stable post-translational modification. They used rat smooth muscle cells as a model to demonstrate that the majority of protein S-nitrosothiols are unstable intermediates, which tend to form disulfides either with other cysteines in the protein or with free glutathione (GSH). Furthermore, they presented evidence that the functions of several known targets of S-nitrosylation are actually affected by disulfide formation rather than directly by $[^{\text{3}}\text{C}]SN^{\text{O}}$. Notably, taken together, these two reports suggest that formation of $[^{\text{3}}\text{C}]SN^{\text{O}}$-mediated disulfides might be the predominant pathway for their anaerobic generation.

Wolhuter et al. (2018) highlight the intrinsic reactivity of NO in biological environments and the remarkable instability of the resulting products. Indeed, post-translational modifications are often viewed as inherently stable, akin to phosphorylation, which requires both kinase and phosphatase enzymes for its genesis and removal. In contrast, some reactions involving NO products do not require direct enzymatic activity. Thus, the resulting modification will, to a large extent, depend on the presence of other reactants, such as glutathione, and on the particular microenvironment of the nitrosylated cysteine. This potentially allows for a much greater dynamic response to environmental changes.

Cysteine residue modification by NO is best viewed in the context of its rates of formation and degradation (Figure 1). The cellular environment specifies one of the kinetic parameters—the rate of NO oxidation to NO$^+$. In the aerobic condition, the hydrophobic protein interior or lipid bilayer tends to accumulate NO levels high enough to promote N$_2$O$_3$ formation (Liu et al., 1998; Nedospasov et al., 2000). Alternatively, the close proximity of electron-accepting transition metals might generate NO$^+$ (Anand and Stamler, 2012; Hickok et al., 2011). For some proteins, specific changes in their activity have been associated with $[^{\text{3}}\text{C}]SN^{\text{O}}$ modification (Seth et al., 2012). S-nitrosylation also causes cysteine residues to become more reactive toward other reduced thiols. Formation of disulfides, S-glutathionylation, and S-cysteinylation, observed by Wolhuter et al. (2018) and others, support this notion (Martinez-Ruiz et al., 2013). Notably, HNO released upon $[^{\text{3}}\text{C}]SN^{\text{O}}$ reaction with a reduced thiol can interact with other cysteines to induce either disulfide or sulfanilamide modification, further affecting protein function (Figure 1). In their report, Wolhuter et al. (2018) clearly demonstrated that disulfides, formed upon $[^{\text{3}}\text{C}]SN^{\text{O}}$ decomposition, change multiple protein functions; however, these reactions would occur in tissue- and cell compartment-specific fashion. In the cytoplasm of cells rich in GSH and thioredoxin (TRX), $[^{\text{3}}\text{C}]SN^{\text{O}}$ that has converted to a disulfide will be quickly reduced to $[^{\text{3}}\text{C}]SH$ (Figure 1). In contrast, in the endoplasmic reticulum, where the GSH/GSSG ratio is very low, $[^{\text{3}}\text{C}]SN^{\text{O}}$ would be much more stable.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{simplified_scheme.pdf}
\caption{Simplified Scheme of Protein S-Nitrosylation, –SNO Propagation, and Regulation of Its Stability in the Cell. Green and orange boxes depict proteins with native and altered activity, respectively. Cys-SH stands for cysteine residue in a protein; GSH, glutathione; TRX, thioredoxin; R, low molecular thiol or cysteine in a protein.}
\end{figure}
GSH reductases by NO lowers the reducing capacity of the cell, concomitantly increasing –SNO stability and disulfide formation by an –SNO-independent pathway (Engelman et al., 2016; Gusarov and Nudler, 2005).

An important caveat exists regarding the choice of NO donor for experimentation. S-nitrosocysteine, used by Wolhuter et al. (2018), induces robust protein S-nitrosylation; however, it preferentially modifies solvent-accessible surface cysteine residues. Newly formed –SNO will be readily available to react with free GSH, producing disulfides. Conversely, NO+ produced by NO auto-oxidation in the protein hydrophobic interior (via micellar catalysis) can S-nitrosylate a very different smaller subset of cysteine residues (Nedospasov et al., 2000). Those –SNOs will be more stable, as they are not readily accessible to GSH. Thus “natural” sources of NO, such as NO-synthase or the effect of anaerobic respiration on nitrate, used by Seth et al. (2018) are more likely to reproduce native protein modifications.

Both reports provide important insights that further our understanding of the formation, propagation and degradation of S-nitrosothiols. The results reported therein call for a critical evaluation of the previous data in order to correctly assign each physiological effect to a specific modification. Better methods need to be developed to study the dynamics and cellular effects of –SNO formation, conversion to other species, and reduction back to –SH.

REFERENCES


