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S-Nitrosation: Current Concepts and New Developments

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Abstract

The S-nitrosation (also referred to as S-nitrosylation) of cysteine residues is an important post-translational protein modification that regulates protein function and cell signaling. The original research articles and reviews in this Forum cover important concepts in protein S-nitrosation and identify key developments and opportunities for progress in this area. Defining the mechanisms by which S-nitrosothiols (RSNOs) may be formed and decomposed in cells and tissues, the integration of the biological chemistry associated with nitric oxide (NO) and other derivatives such as nitrite, and the development of new methodologies merging proteomics and direct quantitation are all key issues that we believe would require detailed attention. *Antioxid. Redox Signal*. 17, 934–936.

Nitric oxide (NO) is a diatomic signaling molecule that regulates many aspects of human physiology and pathophysiology. Studies over the past three decades have uncovered several pathways by which NO acts as a second messenger during signal transduction. Probably, the most characterized and established of these mechanisms is the reaction of NO with the heme center of soluble guanylate cyclase and a consequential increase in the production of the second messenger cyclic guanosine monophosphate (cGMP). More recently, the post-translational modification of cysteine residues in proteins by way of S-nitrosation (also referred as S-nitrosylation) has received increasing attention as a primary mechanism by which NO regulates cell signaling (3). This Forum of *Antioxidant and Redox Signaling* highlights a few of the outstanding key issues and current controversies related to S-nitrosation and signal transduction (see Fig. 1).

Progress in any scientific field is underpinned by technical breakthroughs, allowing specific systems to be probed with increasingly greater details. The subject of S-nitrosation does not escape this point. The review by Bechtold and King details some of the benefits and limitations of current S-nitrosothiol (RSNO) detection methodologies. These include some novel approaches that have recently been developed such as the use of phosphine derivatives (1). In general, RSNOs are inherently unstable, and their detection mostly relies on indirect techniques. This has been an important point of controversy in contrast to other post-translational modifications such as phosphorylation, where immunological and proteomic approaches would appear to be more robust. The most popular methods for RSNO determination include the reductive chemiluminescence and biotin-switch assays. Both methods are based on the removal of the NO group from the RSNOs followed by quantization of NO for the former approach or tagging of the thiol that carried the NO for the latter. Chemiluminescence-based assays provide quantization of RSNOs and have been applied to cultured cells and *in vivo* to examine mechanistic aspects of RSNO formation and their distribution in different organ systems. Obviously, the drawback is the lack of resolution at the protein level, and the biotin-switch assay is the method of choice for proteomic-based approaches. The application of the latter assay has contributed to the exponential increase in studies related to protein S-nitrosation and rapid integration of S-nitrosation (or S-nitrosylation) in a plethora of cell-signaling pathways (7). Real progress in this area will require the merging of the quantitative and proteomic approaches to establish the direct correlation between the extent of protein S-nitrosation and change in protein activity/function.

A careful reading through the literature reveals a sharp contrast between the acceptance of S-nitrosylation as a post-translational modification and the limited mechanistic understanding by which this modification may be controlled *in vivo*. In this Forum, Broniowska and Hogg provide a detailed review of the known mechanisms by which RSNOs may be formed in biological systems (6). Protein-catalyzed S-nitrosation and denitrosation pathways are being uncovered and have to be understood in the context of global proteomic analyses. At the same time, the chemical networks that control RSNO formation and degradation are poorly characterized and have yet to be rationalized with respect to protein specificity and intracellular localization. Even the methodology used to induce a nitrosative environment can influence the experimental outcome. An important illustration of this Forum is provided by Hickok *et al.* (5). They demonstrate that

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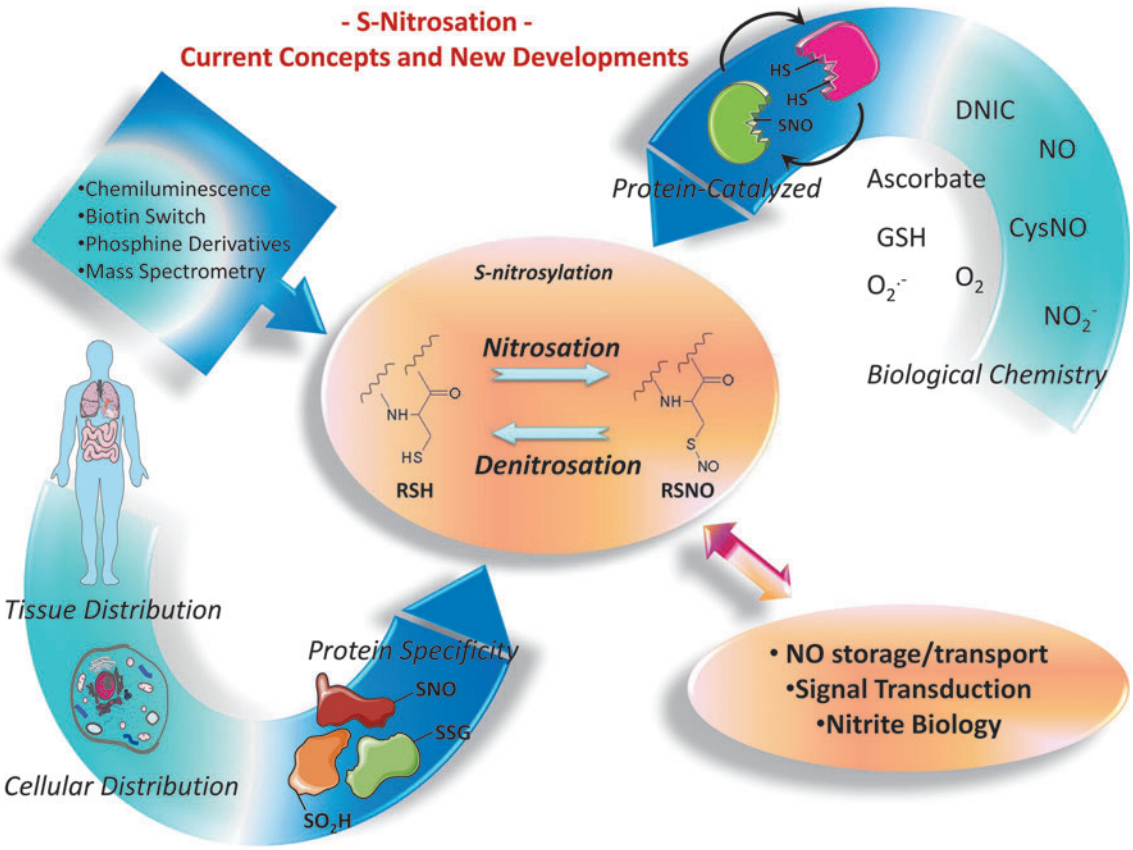

FIG. 1. Protein thiols (RSH) may be modified to form S-nitrosothiols (RSNO) by way of S-nitrosation (or S-nitrosylation). S-nitrosation plays important roles in nitric oxide (NO) transport/storage, signal transduction, and nitrite biology. Several outstanding issues related to S-nitrosation are illustrated in this figure. These include the understanding of the biological chemistry that may control protein nitrosation/denitrosation to integrate the role of reactive oxygen species such as superoxide ($O_2^{\cdot-}$), antioxidants such as ascorbate, and other intermediates such as DNICs. Understanding the tissue and cellular distribution of RSNOs, and protein specificity are also paramount for progress. Lastly, delineating new methodologies that may integrate direct quantitative and proteomics approaches (such as mass spectrometry) are important problems. See text for further details. GSH, glutathione; HS-, thiol; -SNO, nitrosothiol; -SSG, glutathionylated protein; -SO2H, sulfinic acid; RSH, thiol. (To see this illustration in color the reader is referred to the web version of this article at www.liebertpub.com/ars).

great differences in cellular RSNO yields occur when NO or S-nitrosocysteine (CysNO) is used. For studies on protein S-nitrosation, CysNO is most often used as a substitute for NO, but a quantitative analysis of RSNOs subsequent to cellular exposure to either NO or CysNO reveals dramatic differences in the product yields. Dinitrosyliron complexes (DNICs) are the most abundant cellular products from NO exposure, and RSNOs are the major cellular products formed from CysNO. In short, caution should be used before generalization of experimental findings obtained from the sole use of CysNO. A detailed proteomic analysis of the effect of NO and CysNO as well as other NO adjuncts should be conducted to further explore this issue.

RSNOs may not only play a role in regulating nitric oxide synthase (NOS)-derived NO signaling but also play as an important mediator of nitrite bioactivity. Nitrite was long considered an inert molecule in biological systems. However, recent studies have detailed several mechanisms of nitrite activation *in vivo* with a number of physiological and pathophysiological implications such as stimulation of angiogenesis and inhibition of vascular hyperplasia. In the circulation, nitrite-dependent signaling may be mediated through the intermediacy of globin-catalyzed reactions that form RSNOs. In the present Forum, Curtis *et al.* show that in many tissues, nitrite is metabolized to nitrate through the intermediacy of tissue nitrite reductases (2). Interestingly, anoxic nitrite metabolism is accompanied with the formation of RSNOs that is partially dependent on a nitrite reductase activity. Obviously, the implication of RSNO formation during anoxic reduction of nitrite will require additional studies to understand the role that S-nitrosation may play in mediating nitrite signaling. These studies have important implications not only for endogenous nitrite synthesis but also for dietary sources.

A recurrent theme from this Forum is the realization that protein S-nitrosation cannot be adequately interpreted unless the large chemical network associated with NO production is also understood and integrated. For example, it has become evident that DNICs are an important NO reservoir that may be mobilized to form RSNOs. The implication of thiol oxidation in the presence of NO is evident when the coproduction of reactive oxygen species (ROS) in cells and tissues is considered. In this case, S-nitrosation reactions may be blunted

in favor of oxidation. The reaction of NO with molecular oxygen had long been thought to be a primary pathway for S-nitrosation of thiols, yet it is now evident that this reaction might also yield thiol oxidation products in amounts sometimes greater than RSNOs. Overall, this would indicate that many of the signaling effects of NO might be associated with thiol oxidation rather than S-nitrosation. In this Forum, Mongin *et al.* carefully cataloged many protein modifications associated with dysregulation of primary metabolic pathways during overproduction of NO with a focus on neuropathologies (8). For example, in the case of the Krebs cycle, enzymes such as aconitase may be the site of multiple hits including S-nitrosation, S-glutathionylation, oxidation, and tyrosine nitration. Sensitivity to NO may not only be dependent upon the amount and site of NO and ROS production but also be on the relative sensitivity of protein networks to NO and the cellular reliance on these networks. This is most exemplified within the context of the functional integrity of axons and neuronal processes that are critically dependent on local metabolism and timely delivery of metabolic enzymes from the cell body.

The role of extracellular and intracellular antioxidants in RSNO status is even less understood. The tripeptide glutathione is an important redox regulator, and—*in vitro*—glutathione depletion is usually associated with increased protein S-nitrosation. *In vivo*, diseases linked to redox imbalance or antioxidant deficiency are poorly characterized with regard to the RSNO status. This is despite the long-time recognized association of NO with redox dysregulation in many conditions, including cancer, neuropathologies, and cardiovascular diseases. In the present Forum, Garcia-Saura *et al.* provide one such detailed characterization of the RSNO dynamics and distribution in a rat model of ascorbate deficiency (4). This study reveals intriguing relationships between ascorbate status, glutathione levels, and changes in nitroso and nitrosyl products in multiple organs. Although the functional implications of such observations still need to be addressed, the changes in vascular reactivity in response to ascorbate depletion would suggest a role for NO and RSNOs in this response.

In spite of the bourgeoning research related to S-nitrosation and signal transduction, many important questions remain to be addressed (see Fig. 1). The original articles and reviews compiled in this Forum highlight some of the seminal questions facing this field. Central to the significance of S-nitrosation in signal transduction is our understanding of the mechanisms by which protein RSNOs may be formed and decomposed intracellularly. The development of new biochemical and molecular tools for the detection of NO and related nitroso species should facilitate progress in this area. Ultimately, it will become crucial to evaluate whether S-nitrosation is deliberately controlled by a cascade of enzymes that specifically regulate formation and denitrosation, much in the same way as phosphorylation or ubquitination are. Alternatively, S-nitrosation may prove to be a more random event. Perhaps, it functions synergistically with other signal transduction pathways to provide another layer of regulation in a way more comparable to nicotinamide adenine dinucleotide phosphate oxidase-derived signaling. Most likely, we will find that the truth is a delicate balance of both.

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Abbreviations Used

cGMP = cyclic guanosine monophosphate
CysNO = S-nitrosocysteine
DNIC = dinitrosyliron complex
GSH = glutathione
HS- = thiol
NO = nitric oxide
NOS = nitric oxide synthase
ROS = reactive oxygen species
RSH = thiol
RSNO = S-nitrosothiol
-SNO = nitrosothiol
-SO_2H = sulfinic acid
-SSG = glutathionylated protein