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Themed Section: Chemical Biology of Reactive Sulfur Species

EDITORIAL

Highlighted mechanistic aspects in the chemical biology of reactive sulfur species

Correspondence Péter Nagy, Department of Molecular Immunology and Toxicology, National Institute of Oncology, Ráth György utca 7-9, Budapest 1122, Hungary. E-mail: peter.nagy@oncol.hu

Péter Nagy[1], Günter Schwarz[2] and Stanislav Kopriva[3]

[1]Department of Molecular Immunology and Toxicology, National Institute of Oncology, Budapest, Hungary, [2]Institute of Biochemistry, Department of Chemistry, University of Cologne, Cologne, Germany, and [3]Botanical Institute, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Cologne, Germany

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'Life is nothing but an electron looking for a place to rest' a quote by the Hungarian Nobel Laureate, Albert Szent-Györgyi, profoundly emphasizes the widespread and versatile roles of Redox Biology in life on earth. Scientific research in the field was historically focused on the biological roles of oxidant species and free radicals collectively called ROS. The term originates from the recognition that on one hand oxygen is essential for life, while on the other hand, it also serves as precursor of endogenously produced reactive molecules. Although, excessive exogenous or endogenous production of ROS is responsible for several pathophysiological events, today it is also widely appreciated that fundamental biological processes are driven by redox reactions.

Sulfur can acquire a wide range of oxidation states (from −2 to +6), which provides promiscuous redox chemical properties for most sulfur-containing biomolecules making them central players in redox biology. This recognition led to another collective term, reactive sulfur species (RSS), which encompass a plethora of bioactive sulfur compounds ranging from small molecules to peptides and proteins. In the early days, scientific endeavours were dedicated to elucidating the antioxidant properties of RSS, mostly researching the chemical biology of reactive cysteine residues in **GSH** and in proteins with antioxidant functions. The discovery that redox sensing drives cellular adaptation to oxidative stress introduced a new era in the field and diverted the attention toward redox regulation of cellular signalling. Today, sulfur-based redox chemistry is considered an essential coordinating body in many aspects of cell biology, including protein structure, enzyme catalysis, intramolecular gating processes ('redox switches'), cell death signalling, metabolic regulation and ROS scavenging.

The biological roles of low MW RSS acquired substantial importance by the discovery that **hydrogen sulfide (H_2S)** is not only highly toxic to living organisms but also an important endogenously produced signalling molecule with widespread biological functions. H_2S was demonstrated to mediate mechanisms of cellular antioxidant defence as well as redox signalling. However, accumulating evidence suggests that oxidized sulfur metabolites (inorganic- and **cysteine (Cys)**-polysulfides, thiosulfate, tetrathionate, sulfite, etc.) underlie several biological functions that were originally assigned to H_2S. A proposed model by which these low MW RSS drive biological events is *via* inducing persulfide/polysulfide formation on cysteine residues in proteins. Cys-polysulfidation can also be induced *via* other oxidative or enzymic pathways and it was demonstrated to be an important protein-protecting and protein function modulating factor. These ground-breaking discoveries in the chemical biology of RSS introduced a paradigm shift in redox biology and triggered increasing interest in this area.

This themed section addresses important and novel areas in RSS biological chemistry that were largely unmet in the recent sulfur biology literature, such as reprogramming and regulations of trans-sulfuration pathways in light of H_2S production, roles of NADPH driven reducing machineries in redox signalling, translational production of Cys-polysulfides and the chemical biology of oxidized inorganic sulfur species such as polysulfides or sulfite.

Although the mitochondrial intermembrane space (IMS) is a tiny subcellular compartment, it serves a multitude of critical

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roles in a number of cellular processes. A common feature of IMS proteins is conserved cysteine residues with critical biological functions. Habich *et al*. discuss these functions focusing on the redox reactions and post-translational modifications (PTM) of these conserved cysteine residues in relation to cellular metabolism, disulfide bond formation during protein folding and cellular signalling (Habich *et al*., 2019).

ROS-induced oxidation reactions are key in redox regulation; however, reduction reactions mediated by the NADPH-driven thioredoxin and GSH systems are just as important in orchestrating redox events. Miller and Schmidt provided an insightful summary on the essential functions of these reducing processes in a cellular compartment-specific manner, within liver hepatocytes (Miller and Schmidt, 2019). A relatively newly discovered member of the thioredoxin family is the *thioredoxin like protein of 14 kDa* (TRP14). Primary functions of this enzyme are just being elucidated, which surprisingly do not represent cysteine-disulfide bond reducing activities in proteins. Instead, it would appear that TRP14 is largely responsible for the reduction of the amino acid cystine to cysteine after its transport into the cytosol by the **cysteine/glutamate antiporter xCT**. Other recently elucidated TRP14-mediated processes include the reductions of cysteine persulfide and nitrosothiol species. Espinosa and Arnér provide insights into the potential signalling functions of TRP14 *via* these enzymic activities (Espinosa and Arnér, 2019).

The trans-sulfuration pathway is a key cellular component of the metabolism of sulfur-containing amino acids, including **methionine** and cysteine. A relatively recently highlighted action of this machinery is associated with the production of H_2S, which – considering the versatile biological functions of this low MW compound – is gaining increasing attention. H_2S production is mostly proposed to occur *via* reverse trans-sulfuration, meaning that trans-sulfuration enzymes use cysteine to produce H_2S. (The term trans-sulfuration is derived from the recognition that these pathways catalyse the conversion of methionine sulfurs to cysteine production.) Kohl *et al*. provide a comprehensive overview on the catalytic pathways of cysteine catabolism leading to sulfite and thiosulfate as terminal products. They discuss different homeostatic effects of sulfite and H_2S on cysteine metabolism as well as the potential clinical impacts of their malfunctioning (Kohl *et al*., 2019). Related to this, Kimura *et al*. suggested that sulfite (a terminal oxidation product of H_2S and cysteine) can protect neurons from oxidative stress *via* enhancing intracellular cysteine (and GSH) levels (Kimura *et al*., 2019). They proposed that sulfite can facilitate the extracellular reduction of **cystine** to cysteine, for which intracellular transport is facilitated and not retarded by inhibitors of the cysteine transporter xCT, such as **glutamate**. Regulation of trans-sulfuration pathways has pivotal roles in sulfur biology. Sbodio *et al*. contributed with an insightful summary on these regulatory pathways and their malfunctioning (Sbodio *et al*., 2019). The authors discuss these mechanisms in relation to the pathophysiological consequences of dysregulated trans-sulfuration and provide novel strategies to tackle associated diseases. Kozic *et al*. conducted a comprehensive analytical investigation on the trans-sulfuration metabolome in blood and urine samples of patients that either suffer from severe deficiency of the trans-sulfuration enzyme **cystathionine β-synthase (CBS)** or have re-methylation defects (Kožich *et al*., 2019). The study came to the surprising conclusion that these severe inherited defects in sulfur amino acid metabolism may only moderately perturb H_2S metabolism, implying either high levels of redundancy or alternative routes of primary H_2S synthesis.

Fujii *et al*. summarize recent discoveries suggesting that Cys-polysulfide species can be produced translationally by moonlighting functions of cysteinyl t-RNA synthase 2 (CARS 2) (Fujii *et al*., 2019). Furthermore, the essential roles of the Cys-polysulfides, produced by the CARS 2 pathway, in mitochondrial bioenergetics propose a novel role for these species as integrated elements in respiratory processes.

Post-translational productions of cysteine polysulfides include oxidation of protein cysteine thiols with inorganic polysulfide species. The chemical properties, biosynthesis and available detection methods for inorganic polysulfides are discussed by Liu *et al*. (2019). Pozsgai *et al*. focus on the effects of inorganic polysulfides and sulfide on **TRP cation channels**). They discuss direct interaction-based mediation of protein functions as well as indirect regulation by RSS *via* modulating a wide range of signalling pathways related to TRP channel activities (Pozsgai *et al*., 2019).

Considering the wide-ranging and fundamental functions of RSS in biology, it is essential to have both reliable protocols to detect their speciation in biological systems and pharmacological tools to study their physiological roles. Bogdándi *et al*. conducted a comprehensive analytical study to demonstrate that alkylation-based protocols can substantially change speciation of RSS representing a major caveat for currently available cysteine PTM-detecting protocols (Bogdándi *et al*., 2019). Importantly, in light of the fact that current state of the art methodologies all use an alkylation step or consume RSS in other irreversible reactions, the authors do not suggest these protocols should be excluded, but raise important issues that need to be kept in mind when interpreting results obtained with these techniques. Utilizing these observations, Bianco *et al*. demonstrated that cystine trisulfide (CysSSSCys) can be efficiently taken up by cells, providing a useful tool to pharmacologically induce polysulfide formation on proteins and small molecules in the intracellular matrix (Bianco *et al*., 2019). Rigorous chemical understanding of these technologies and their limitations is essential in order to properly integrate them into whole animal and clinical studies, which will lead to better understanding the roles of RSS in health and disease.

Altogether, this themed section documents recent advance in sulfur redox research and the recognition of complex sulfur compounds as drivers of many regulatory processes. The number of novel knowledge-based questions and challenges identified and presented in individual papers guarantee that the chemical biology of RSS will continue to be a dynamic and exciting field of research.

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Conflict of interest

The authors declare no conflicts of interest.

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