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Connecting the dots

New tools and rigorous chemical and biological studies will be essential for enhancing our mechanistic understanding of cellular oxidative stress.

In the past decade, we have been urged to enrich our diets with foods and supplements containing 'antioxidant' compounds that have been reported to protect us from the effects of damaging free radicals and lower our risk of disease. Though such claims have allowed many of us to enjoy more chocolate or another glass of red wine in the name of better health, the scientific mechanisms behind these claims are not so simple. Oxidative stress is an established feature of aerobic cells, but our understanding of the complex and interconnected set of reactions that generate reactive species and mediate their biological effects remains incomplete. Further molecular understanding of oxidative stress mechanisms and our ability to manipulate these pathways will require an interdisciplinary effort that combines robust chemical thinking and tools that are calibrated by relevant biological data and insights.

Oxidative stress occurs when the redox balance within the cell is perturbed in favor of oxidizing species. For decades, oxidative stress research has focused on understanding how proteins, lipids and DNA are damaged by reactive species. Though numerous factors, including environmental conditions and endogenous metabolism, can trigger oxidative stress, the molecular effectors are typically few, including reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl radicals ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2), or reactive nitrogen species (RNS) such as nitric oxide (NO) and its metabolites. In addressing oxidative stress, it has become tempting to see antioxidants as a panacea. Yet it is incorrect to view all oxidative chemistry as deleterious: for example, these same reactive agents are harnessed by the immune system to target microbial infections. More recently, ROS and RNS have been assigned roles as signaling molecules that interact with key sensor proteins and couple oxidative signals to biochemical pathways. In contrast to most small-molecule metabolites that exert their biological effects by noncovalent binding to a receptor, ROS and RNS signals generally act via the covalent modification of their cellular targets to produce post-translational modifications. The involvement of ROS and RNS in such diverse pathways underscores the need for an enhanced understanding of the biological roles of these reactive chemical species.

Elucidating the molecular mechanisms of oxidative stress and signaling pathways remains a challenging scientific pursuit. The primary hurdle is that oxidative reactions are inherently complicated: they depend on a highly complex and interdependent set of chemical reactions that connect with biomolecular substrates under cellular conditions. Unfortunately, the direct detection of short-lived ROS or RNS also presents a major technical challenge, which makes it difficult to measure the concentration or localization of these intermediates. Furthermore, the kinetic barriers for transformation of ROS and RNS into other reactive intermediates are generally low, and so one-to-one mapping of a specific reactive agent to a particular biological effect is not straightforward.

These systematic and technical challenges are complicated by the variable levels of mechanistic rigor that are applied by researchers studying oxidative reactions. A review article in this issue by Christine Winterbourn (p. 278) asks us to reexamine our understanding of the chemical and biological roles of ROS. As she discusses, we need to apply greater chemical rigor to mechanistic hypotheses related to oxidative stress. Chemists have characterized the reactivity of ROS and RNS in solution, and these kinetic and thermodynamic parameters form a basis set that describes the fundamental chemical reactivity of these species. It is imperative that researchers carefully consider this chemical knowledge when they propose and test oxidative mechanisms in cells. On the other hand, though these chemical model systems provide an indispensable starting point for analyzing cellular oxidative stress, they do not take into account the more complex environments and components of cells. Thus, we must also require greater biological rigor in studies that probe ROS and RNS reactivity in cells.

Chemical biologists have been leading efforts to understand oxidation in biological systems. Indeed, they have already made significant strides in tackling one of the main challenges facing the field: the development of methods for quantitative detection of ROS and RNS in cells (for an NO sensor, see *Nat. Chem. Biol.* **2**, 375–380, 2006; for an H_2O_2 sensor, see *Nat. Chem. Biol.* **3**, 263–267, 2007). There is also a need for methods that permit the identification of proteins that undergo post-translational modifications in response to ROS or RNS, such as a recent technique for detecting sulfenic acid modifications (*Mol. Biosystems*, published online 14 March 2008, doi:10.1039/b719986d; highlighted on p. 277). Finally, chemical genetics may offer a powerful approach for probing and modulating biological responses to oxidative stress (*Nat. Biotech.* **26**, 343–351, 2008; highlighted on p. 277). Ongoing methodological innovation will provide tools to answer the broader questions of how oxidative chemistry affects and is integrated into biological systems.

Beyond providing technological advances to move the field forward, chemical biologists can support research into biological oxidation in other ways. They should ensure that researchers are trained with the breadth and depth of knowledge necessary to understand the chemistry and biology of complex systems such as redox biochemistry (see p. 267). Chemical biologists interested in oxidative stress should also use their familiarity with interdisciplinary research to better integrate concepts and experimental approaches from chemists, biologists and toxicologists. As we approach the biology of ROS and RNS with new tools, chemical rigor and biological insights, perhaps we will be able to indulge in that chocolate soufflé or glass of wine with greater confidence, or at least greater knowledge. ■