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Forum Editorial

Oxidative Modification of Proteins in Cell Signaling

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CHANGING PERSPECTIVES: OXIDATIVE STRESS VERSUS OXIDATION WITH A PURPOSE

SCIENTIFIC PARADIGM SHIFTS seldom occur overnight, except when considered retrospectively, long after the fact. The history of free radical and oxidative stress research during the past century has been a case of constantly evolving ideas with several major philosophical redirections that qualify as true paradigm shifts (5). The most recent of these has been occurring since the late 1980s and has come to full fruition only in the past several years. By the mid to late 1980s, a relatively small number of pioneering labs had more or less proved that oxidative stress was a real phenomenon that likely contributes to aging and disease (for review, see 5). However, prior to the surprise discovery of nitric oxide (•NO) as a signaling molecule (6, 8, 14), oxidative stress was considered as a purely pathophysiological phenomenon. With the identification of •NO as "endothelium-derived relaxing factor" (6), two truths became incontrovertible: (a) free radicals are not only real, but essential entities in mammalian biochemistry; and (b) reversible protein oxidations (at least those affected through •NO) are often a routine, purposeful aspect of a cell's normal business operations, rather than a totally stochastic consequence of oxidative metabolism.

The explosion in research triggered by discovery of •NO resulted in a more enlightened consideration of reactive oxygen and reactive nitrogen species (ROS and RNS). Many studies conducted over the past decade have focused on whether, and how, molecules such as superoxide radical anion (O_2•$^-$) and its disproportionation product hydrogen peroxide (H_2O_2) might act as second messengers to regulate "classical" signal transduction pathways. Researchers interested in protein kinase cascades, G protein-coupled receptors, and the like began to consider how these molecular conduits for bioinformation might react to oxidative stress. Several key studies in the mid-1990s clearly demonstrated that H_2O_2 is a ubiquitous second messenger, rapidly generated in multiple cell types upon specific cell surface receptor activation events, leading to accentuation of protein kinase cascades (7, 12, 16). Sources for these ROS were identified and found to include both mitochondria and membrane-associated NADPH oxidases (for review, see 3) that could be recruited rapidly following appropriate ligand-receptor recognition. Subsequent key experiments implicated protein tyrosine phosphatases as exquisitely vulnerable targets for endogenously generated peroxide and other electrophiles, undergoing reversible and irreversible types of oxidative modification (2, 3, 12). Exploration of reactive oxygen signaling was accelerated by interest in mitogen-activated protein kinases and the nuclear factor-κB pathways that act in a reciprocal fashion with cognate redox-sensitive phosphatases to control diverse aspects of cell viability, mitosis, and gene expression (2, 3, 12, 15).

Despite the correlations that were repeatedly observed between ligand-stimulated ROS generation and concomitant signal transduction activities (*e.g.*, effector enzyme activation), the question remained as to whether ROS generally serve as necessary modulators of signal transduction, as opposed to being an accidental epiphenomenon, a type of unavoidable metabolic artifact that introduces noise into the otherwise well orchestrated signal transduction machinery. To some extent that question remains open to debate. Most cell biologists would now accept that •NO is not unique among ROS in its role as a purposeful second messenger. The evidence to the contrary, although often circumstantial, is nonetheless compelling.

That evidence is reviewed and reinterpreted in the articles collated within this *Forum*. We now understand, in many cases, how peroxide generation is coupled to protein thiol oxidation and how the process is reversed during the course of ligand-stimulated signal amplification processes (1, 11, 13). The mechanisms responsible for this redox cycling are so well orchestrated that it is more difficult for one to imagine that the ROS generation is an unavoidable accident than it is to hypothesize models for purposeful redox signaling. Indeed we now recognize that protein tyrosine phosphatases can adopt as many as five discrete oxidation states (thiolate, sulfenic acid, glutathionyl disulfide, cyclic sulfenamide, and sulfinic acid) that facilitate redox tuning of the kinase:phosphatase junction while avoiding irreversible damage to the

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proteins in question (13). These various oxidation states are accessible and stable enough to have been trapped in crystallographic structures (13). It is especially informative to compare and contrast the known roles for H_2O_2 in redox signaling with the action of •NO (4). Although both H_2O_2 and •NO react with protein thiols, the specificity, kinetics, and mechanisms for reversibility are very different (4). Thus, the cell has a rather flexible set of redox tools to call upon for dealing with its changing needs.

The very subtlety of redox signaling, as revealed and elaborated upon in these *Forum* contributions, is a caveat to the cell biologist studying redox issues, as well as an inspiration to the scientist studying pathophysiology. Although ROS and RNS may be necessary substances to effectively convey biological signals, it is very easy to exceed a cell's tolerance limits and derange its redox signaling pathways. Several of the studies in this *Forum* describe the consequences of redox aberrations, such as might be induced under conditions of hyperglycemia (17), ischemia/reperfusion injury (10), or folate cycle compromise (18). Thus, a careful experimentalist must discriminate whether his systems address a normal or a pathophysiologic region of the redox landscape. Finally, we are reminded by Pfannschmidt *et al.* (9) that mammalian cells have no monopoly on lessons they can teach us about redox signaling; there is much to glean from comparative considerations of mammalian and plant pathways for redox signal transduction, particularly with respect to mechanisms of genetic regulation.

FUTURE DIRECTIONS

We are further challenged by these contributions to renew our search for molecular targets of endogenously generated ROS/RNS that discriminate amongst superoxide, H_2O_2, and $[NO]_x$. Along the same lines of inquiry, more effort is needed to develop new systems models for redox integration: ROS are intrinsically more promiscuous in their reactivity than most classical second messengers, so that a given transient of ROS is likely to affect a greater number of target enzymes than would a given transient of cyclic AMP or Ca^{2+}. Thus, the classical deterministic models will not suffice to adequately describe systems in which redox signaling is relevant. There are likely other major opportunities remaining to identify the fine mechanisms by which ROS are generated consequent to specific ligand:receptor activation events; for instance, we know much about how NADPH oxidases assemble from their constituents, but we have little detailed molecular knowledge of how mitochondria regulate oxidant release into the cytosol during redox signal transduction. Finally, we are reminded of the ultimate challenge: how are we to use our growing knowledge of redox signaling to design improved strategies for promoting human health and wellness? It is not obvious how this might be accomplished, other than by flooding the body with phenolic or thiol-based "antioxidants," which might inadvertently interfere with normophysiologic redox processes. These questions will likely stimulate biochemical investigations for decades to come as our paradigms continue to evolve.

ABBREVIATIONS

H_2O_2, hydrogen peroxide; •NO, nitric oxide; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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