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Redox Sensing: Novel Avenues and Paradigms

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Abstract

The response to changes in the redox state of the cell environment is closely coupled with the ability of living organisms to sense changing conditions. Protein-based redox sensors utilize cofactors, that is, iron–sulfur clusters, flavins, or hemes, for environmental sensing. Under oxidizing conditions a cofactor-mediated post-translational modification (*i.e.*, thiol-oxidation, carbonylation, or dityrosine formation) accompanied by a structural change in the protein occurs that results in an appropriate reaction, mostly in terms of expression of genes involved in antioxidative stress responses. In addition to these well-studied cofactors, researchers have recently discovered and described redox-active metabolites that play a role in redox sensing. Furthermore, not only proteins but also nucleic acids are able to sense redox-stressing events and to elucidate the corresponding response. With these all sensors, organisms are well equipped to sense redox-stress signals generated extracellularly as well as cytoplasmatically. To analyze the molecular mechanisms of all these redox sensors as well as to describe the paradigms involved, a number of sophisticated tools have been applied. These include development of novel protein fluorescence resonance energy transfer probes to microscopically analyze redox signaling in cells or the application of X-ray crystallography combined with spectroscopic studies to monitor dynamics of conformational changes within redox sensors. In this Forum, novel redox-sensing systems, novel avenues, and recent technical advances in the emerging field of redox sensing are presented. *Antioxid. Redox Signal*. 16, 636–638.

Do all stressors have a negative connotation? In this Forum, Okegbe *et al.* address this question emphasizing that the response to a stressor can be positive (eustress) or negative (distress) (6). This dual nature of stress is often confusedly used by some researchers, as they typically consider stress as an agent inducing pathology in a particular biological context, thus leading to a disturbance in homeostasis. In different cases, however, the organism is not negatively affected by exposure to the stressor. For instance, the redox-cycling metabolite phenazine has been long considered only as an antibiotic, as it generates reactive oxygen species (ROS) leading to cell death in some organisms (distress). They have, however, a large number of beneficial roles (eustress) that have been extensively studied in *Pseudomonas aeruginosa* (2). Okegbe *et al.* provide a detailed analysis of the mode of action of phenazines, including their role in iron acquisition, in modulation of community development, as signaling molecules, in respiration and redox balancing. Another described redox-cycling compound, having the dual character of a stressor described by (6), is nitric oxide. This compound and phenazines can activate the activity of the redox sensor NsrR or SoxR, respectively, *via* the modification of their iron–sulfur (Fe-S) clusters.

Recently, another Fe-S cluster–containing protein, named WhiB3, has emerged as a redox sensor and effector molecule, controlling several aspects of the virulence of *Mycobacterium tuberculosis* (*Mtb*) (9). WhiB3 specifically reacts, *via* its 4Fe-4S cluster, with important host gases (O_2 and NO), and exogenous and endogenous metabolic signals to maintain redox balance. In this Forum, Saini *et al.* emphasize the importance of maintaining *Mtb* redox balance, as the bacilli encounters a large number of redox stressors during infection, and because front-line anti-*Mtb* prodrugs (isoniazid, ethionamide and PA-824) require bioreductive activation to exert antimycobacterial effects. Although molecular mechanisms governing the function of WhiB3 have not been elucidated in detail, its involvement in diverse range of biological events, including redox homeostasis, cyclic adenosine monophosphate regulation, oxidative stress, cell division, drug resistance, and virulence, was demonstrated. In particular, its role as a metabolic redox sensor in host cells has a high relevance (9, 11). Moreover, WhiB3 is a DNA-binding protein. It was shown that the oxidation state of Fe-S clusters has an effect on DNA binding and hence on the WhiB3-dependent transcriptional activity. In addition, cysteine residues appear to play an important role in the transcriptional regulation, *via* oxidation of thiol groups (9).

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Thiol groups of redox-sensitive cysteines are highly susceptible to oxidation by ROS. Thus, cysteine-containing proteins are ideal candidates to sense redox changes by using sensory thiols (12). In this Forum, Wang *et al.* define ROS as input, and the conformational changes and/or the other post-translational modifications (*i.e.*, phosphorylation, acetylation, ubiquitination, and SUMOylation) after thiol oxidation are the output of redox sensing. In this paradigm, the effectors are the same or related proteins, their abundance, half-life, localization, and activity can be altered. The authors focus the review further on the redox-sensing proteins Sentrin/SUMO-specific protease 3 (SENP3) and caspase-9. SENP3 is a SUMO2/3 protease that has been reported as a novel client protein of the chaperone heat shock protein (Hsp90) and the co-chaperone/ubiquitin ligase carboxyl terminus of Hsc70-interacting protein (CHIP) (13). While under nonstressing conditions the level of SENP3 is maintained at a basal level, under mild oxidative conditions it undergoes thiol modification leading to an interaction with Hsp90. In the Hsp90/SENP3 complex SENP3 is protected from CHIP-mediated degradation. This is a novel type of interaction of CHIP and Hsp90 with a client protein that depends on the oxidation state of the client (12, 13). Caspase-9, the initial caspase in the mitochondrial apoptotic cascade, also undergoes cysteine oxidation, leading to the formation of intra- or intermolecular disulfide bonds that regulate its function (12).

Other ROS-mediated oxidative modifications in sensory proteins include the formation of carbonyl derivatives and dityrosines, which have been detected in the extracellular redox sensor HbpS upon iron-mediated stress (8). The octameric HbpS acts as an accessory module that regulates the activity of the two-component system SenS-SenR. Thus, HbpS-SenS-SenR has been defined as a three-component system. Moreover, it is considered to be a model system for the corresponding homologs in other bacteria with ecological, medical, and biotechnological relevance (10). In this Forum, Siedenburg *et al.* review the *in vivo* and *in vitro* characterization of HbpS-SenS-SenR, which was achieved employing different tools of molecular genetics, microbiology, biochemistry, and structural biology. Oxidative stress-mediated modifications in HbpS are accompanied by intrinsic overall conformational changes, resulting in the up-regulation of HbpS-Sen-SenR. In this Forum, Klare and Ortiz de Orué Lucana report data showing dynamics of conformational changes of HbpS subunits within the octameric assembly. To demonstrate these dynamics, the authors used site-directed spin labeling combined with pulse electron paramagnetic resonance (SDSL EPR) spectroscopy (5). They emphasize that SDSL EPR has been used in their presented work for the first time to monitor redox-mediated conformational changes in a sensory protein in solution. Moreover, they report further physiological and biochemical data showing that a mycelia-associated catalase-peroxidase, under the control of HbpS-Sen-SenR, protects HbpS *in vivo* from oxidation. Thus, freshly synthesized and unmodified HbpS down-regulates the activity of HbpS-SenS-SenR under nonoxidizing conditions (5, 10).

In analogy to iron-mediated conformational changes in HbpS, other sensors utilize a prosthetic group to sense gases, that is, O_2 and NO, that modulate their activity. In this Forum, Aono reviews X-ray crystallographic and spectroscopic (*i.e.*, UV/Vis, Raman, EPR, and Mössbauer) studies revealing that the interaction between a prosthetic group, namely, heme or Fe-S cluster, and gas molecules triggers dynamic structural changes in the protein backbone (1). For instance, the heme-containing fumarate and nitrate reduction (FNR) transcriptional regulator, controlling the expression of >100 genes, utilizes Fe-S clusters as active sites for the sensing of O_2. Under anaerobic conditions, FNR is a homodimer with each monomer containing a $[4Fe\text{-}4S]^{2+}$ cluster, which is O_2 labile and is converted into a $[2Fe\text{-}S]^{2+}$ cluster on exposure to O_2. A prolonged exposure to O_2 results in the loss of the cluster, leading to the formation of the apo-form of FNR. This provokes a dissociation of FNR into monomers, thereby losing specific transcriptional activity (1). The author emphasizes that the detailed mechanism of this cluster conversion has been recently clarified. In heme-based sensor proteins, implicated in signal transduction cascades, gas molecules are sensed by reversible binding to heme. In these cases conformational changes are induced by the reorganization of hydrogen-bonding networks between the heme-bound gas molecule and the surrounding amino acid residues, which also play a crucial role in gas discrimination and subsequent signal transduction.

Not only *in vitro* but also *in vivo* techniques were continuously developed to investigate redox stressing events in living cells. In this Forum, Oku and Sakai present redox-sensing fluorescent proteins that usually utilize their characteristic redox-sensing domains as linkers in between two fluorophores, where structural alternations of the domains lead to changes in fluorescence resonance energy transfer (FRET) efficiencies across the fluorophores (7). The use of such redox protein probes in live imaging within a cell provides an opportunity to monitor redox-dependent changes under physiological conditions (*i.e.*, during developmental, pathogenic, and aging processes). The authors describe molecular mechanisms underlying the functions as well as biochemical properties of two types of novel FRET-based redox probe proteins, namely, RL-derived probes and Redoxfluor. Notably, these redox probes were found to directly respond to the redox state of glutathione, and thus provide a promising property for their use in subsequent *in vivo* analyses (7). Moreover, Redoxflour can be used not only in the cytoplasm but also for sensing intra-mitochondrial redox state. Furthermore, the authors introduce the application possibilities of these probes to *in vivo* analyses.

Sensing of oxidative stress is not restricted only for proteins. In this Forum, Siedenburg *et al.* summarize processes in which nucleic acids play a central role in redox sensing. ROS-mediated oxidation of nucleic bases can result in the generation of radicals (*i.e.*, guanine radicals) that can induce a so-called charge transfer or electron migration. One DNA-based protection mechanism appears to include the transport of charges across the DNA, facilitating the transport of a mutagen (guanine radical) from more important (coding) to less important (noncoding) DNA regions (4). A second mechanism implies a DNA-mediated oxidation of a transcriptional regulator, which in turn activates expression of genes involved in anti-oxidative stress response (3). Small regulatory RNAs, the so-called riboswitches, appear to be also implicated in redox sensing. Riboswitches regulate gene expression, at transcriptional or translational levels, in a ligand-dependent manner. In this context, redox-active metabolites and metal ions have been identified as riboswitch ligands. The studies presented by Siedenburg *et al.* provide interesting details for likely a new avenue in the field of redox sensing, namely, redox sensory RNA (10).

Redox sensing is a continuously emerging field. In future and using genome- and metabolome-related technologies, currently nondescribed pathways and metabolites will likely be identified. The use of more elaborated tools of molecular genetics, biochemistry, structural biology, and cell imaging will additionally help to clarify their physiological relevance and molecular mechanism of function.

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Abbreviations Used

CHIP = carboxyl terminus of Hsc70-interacting protein
EPR = electron paramagnetic resonance
Fe-S = iron–sulfur
FNR = fumarate and nitrate reduction
FRET = fluorescence resonance energy transfer
Hsp90 = heat shock protein
Mtb = *Mycobacterium tuberculosis*
ROS = reactive oxygen species
SDSL = site-directed spin labeling

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