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FORUM EDITORIAL

Intracellular Redox Compartments: Mechanisms and Significances

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Abstract

With the appearance of oxygen and the development of aerobic life on earth, reactive oxygen species (ROS) became important factors influencing a number of processes within a cell. Although initially considered unwanted and harmful by-products of a number of cellular reactions, the last decade has shown that ROS can also act as signalling molecules mediating changes in O_2 tension, as well as the response to hormones, growth factors, and mechanical or chemical stress. Different ROS-generating and ROS-degrading systems in different intracellular compartments seem to play an important role. In line with this, it appears that proteins already well known for an ROS-unrelated specific function in one compartment participate in the ROS response within another compartment. Thus, it is easy to envision that redox changes in different compartments and resulting changes in ROS levels may represent an important mechanism of intracellular communication between different cellular compartments. *Antioxid. Redox Signal.* 13, 395–398.

With the appearance of oxygen and the development of aerobic life on earth, reactive oxygen species (ROS) became important factors influencing a number of processes within the cell. In mammalian cells, ROS can be formed in response to toxic reagents or as by-products of O_2-using enzymes such as those in the respiratory chain, the arachidonic acid pathway, the cytochrome P450 family, glucose oxidase, amino acid oxidases, xanthine oxidase, NADH/NADPH oxidases, or NO synthases (5, 14). Because eukaryotic cells display a characteristic compartmentalization into organelles, which all have a unique structural and functional identity, it is likely that each compartment contributes to the overall ROS production or degradation. Superoxide anion radical (O_2^{-}•) formation is often the initial step in ROS generation, which then participates in the generation of other ROS: most important, hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), peroxynitrite ($ONOO^{-}$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$) (10).

Because large amounts of ROS can cause damage to proteins, DNA, and lipids, cells use an array of nonenzymatic and enzymatic detoxification mechanisms to cope with ROS. Under physiologic conditions, excess formation of ROS is prevented by the endogenous antioxidant defense systems. These include superoxide dismutases (SODs), glutathione peroxidases (GPXs), catalase, thioredoxin peroxidases (peroxiredoxins), glutaredoxins, the thioredoxin–thioredoxin reductase system, and exogenously taken up micronutrients and vitamins (3, 15). In addition, several redox systems like the NAD^+/NADH, $NADP^+$/NADPH, and oxidized glutathione/reduced glutathione (GSSG/2GSH) contribute to redox homeostasis.

A number of laboratories including our own have shown over the last decade that, in addition to the harmful effects of ROS, ROS can also act as signalling molecules mediating changes in O_2 tension as well as the response to hormones, growth factors, and mechanical or chemical stress. Thereby, different ROS-generating and ROS-degrading systems in different compartments of the cell seem to play an important role. In line with this, it appears that proteins already well known for an ROS-unrelated specific function in one compartment participate in the ROS response within another compartment. Thus, it is easy to envision that redox changes in different compartments and resulting changes in ROS levels may represent an important mechanism of intracellular communication between different cellular compartments. Interestingly, almost all cellular compartments and even the extracellular space have been shown to contribute to either ROS generation or degradation with intracompartmental systems, which are not known to the last detail. Therefore, the current FORUM ISSUE, which comprises a series of review articles and original manuscripts, highlights the current knowledge and important new findings related to different aspects of cell signalling in response to ROS-modulating systems in different cellular compartments.

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In their review, Chaiswing and Oberley (7) discuss that the extracellular redox (reduction–oxidation) state can be determined by several redox-modulating proteins that are located on the plasma membrane or outside of cells. These molecules contribute to generation and degradation as well as the travelling of ROS and reactive nitrogen species (RNS) across the plasma membrane. The resulting influx and efflux of ROS may then especially be important under pathologic conditions such as cancer. The extracellular redox state may be altered, which then may cause specific proteins such as proteases, soluble factors, or the extracellular matrix to have altered functions or activities. A number of findings have shown that the function of ROS-generating NADPH oxidases, subunits of which can partially be found or be recruited to the plasma membrane, can be affected by signals triggering the activation of integral plasma-membrane receptors. Although NADPH oxidase was initially described as the respiratory-burst enzyme in neutrophils, recent studies revealed the existence of a family not only confined to neutrophils but also active in various cell types (22). In addition, the GTPase Rac, which plays an important role in the regulation of growth-factor signalling, plays an important role in modulating the activity of these enzymes, thus linking NADPH oxidase function to growth-factor signalling. The review by Petry *et al.* (25) describes the complex network of NADPH oxidase regulatory pathways with emphasis on the activation by different membrane-bound receptors. In view of this, it appears that redox buffer networks in the extracellular space or at the plasma membrane may possess the potential for therapeutic interventions in cancer.

In addition to cancer, it has been shown that mitochondrial proteins appear to be major targets of oxidative damage during aging. Because mitochondria are a major source of reactive oxygen species, mitochondrial proteins are especially exposed to oxidative modification, and elimination of oxidized proteins is crucial for maintaining the integrity of this organelle. Recent studies reported that the ATP-stimulated Lon protease within the mitochondrial matrix contributes to the degradation of oxidized proteins. In their review, Ugarte *et al.* (26) address the role of the mitochondrial Lon protease and the oxidized protein-repair system methionine sulfoxide reductase in the context of oxidative stress and aging. Whereas the age-associated impairment of Lon-like protease activity contributes to the buildup of oxidized proteins in the mitochondria, other proteins, whose function, apart from ROS signalling, was thought to be completely understood, were also found to be present in the mitochondria. Among these proteins are the transcription factor signal transducer and activator of transcription 3, Src kinase family members, Src, Fyn, and Yes, the tyrosine phosphatase Shp-2, and telomerase reverse transcriptase, the key enzyme preventing telomere erosion in the nucleus. The review by Büchner *et al.* (4) discusses the unexpected localization of these proteins in mitochondria, along with their mitochondrial or nuclear functions (or both), which add a new layer of complexity in ROS signalling. The flexibility to cope with ROS extends also to other organelles like the endoplasmic reticulum (17) and the lysosome. The review by Kurz *et al.* summarizes recent findings indicating that the lysosome is a redox-active compartment (21). Although Fenton reactions were first localized to the endoplasmic reticulum (23), it appears that the acidic milieu and the high concentration of thiols will keep reduced the low-mass iron and copper that are liberated after degradation of metalloproteins. This will then allow intralysosomal Fenton reactions. These reactions may contribute to formation of lipofuscin or "age pigment." Many malignancies are characterized by increased amounts of iron- or copper-containing macromolecules that are turned over by autophagy, and thus it is conceivable that the lysosomotropic iron or copper chelators may be used as therapeutics in age-related diseases or cancer.

However, age-related problems and disturbances in the function of the central nervous system, such as seen in Zellweger syndrome and X-linked adrenoleukodystrophy, were also found to be associated with defects in the peroxisome. Peroxisomes are multifunctional organelles with an important role in the generation and decomposition of reactive oxygen species (ROS). In their review, Antonenkov *et al.* (1) summarize the current knowledge and growing evidence showing that tissue-specific abnormalities in peroxisomal ROS metabolism can lead to liver and kidney failure, heart disease, as well as inflammatory changes in the nervous system. The functional plasticity of mammalian peroxisomes, which are mainly under the control of the transcription factors from the peroxisome-proliferator–activated receptor (PPAR) family, provides an opportunity for the development of therapeutic applications directed toward the cure of diseases caused by peroxisomal dysfunction.

The involvement of PPARs links peroxisomal ROS production to the nuclear transcriptional machinery. The nucleus itself contains a number of proteins with oxidizable thiols that are essential for transcription, chromatin stability, nuclear protein import and export, as well as DNA replication and repair. These processes depend on the common thiol reductants, glutathione (GSH) and thioredoxin-1 (Trx1). Recent evidence shows that these systems are controlled independent of the cytoplasmic counterparts. In the review by Go and Jones (16), recent aspects of these specific control mechanisms for nuclear thiol-disulfide redox states are discussed. Because specific isoforms of glutathione peroxidases, glutathione *S*-transferases, and peroxiredoxins are enriched in nuclei, this suggests that functions of the thiol-dependent systems in nuclei are distinct from similar processes in the cytoplasm and may directly affect the function of transcriptional regulators.

Several transcription factors have been thought to be involved in the redox-dependent modulation of gene expression. Among these, nuclear factor κB (NF-κB), activator protein 1 (AP-1), the promoter-specific transcription factor Sp1, and the hypoxia-inducible factor-1 (HIF-1) appear to play central roles. The importance of HIF-1 has been underlined by findings showing that the α-subunit of HIF-1 (HIF-1α) induces >100 genes (24), among them, those encoding proteins that are involved in regulating key aspects of the tumor cell phenotype.

Although HIF-1 was originally identified to be induced and activated only under hypoxia, accumulating evidence indicates that HIFs play a more general role in the response to a variety of cellular activators and stressors. The α-subunit of HIF-1 (HIF-1α) is also responsive to different stimuli, including growth factors and prothrombotic factors, inflammatory cytokines, as well as reactive oxygen species (ROS) under nonhypoxic conditions [reviewed in (20)]. In addition to HIF-1α, two other HIF α-subunits have been identified: HIF-2α and HIF-3α. HIF-1α and HIF-2α have been clearly established to be

responsive to hypoxia. This is primarily brought about by the regulation of protein stability and coactivator recruitment and involves hydroxylation reactions carried out by specific prolyl and asparaginyl hydroxylases [reviewed in (19)]. In addition to the O_2-dependent hydroxylation reactions that enable binding of the VHL (von Hippel-Lindau) tumor-suppressor protein and subsequent proteasomal degradation, it was found that HIF-1α can be modified by Ref-1 (18) and can interact with coactivators such as CBP/p300, TIE-2, and SRC-1 in a redox-dependent manner (6, 13). However, the underlying regulatory mechanisms and signalling events with which superoxide and derived ROS control HIFs are only slowly beginning to be elucidated.

Because the NADPH oxidase activator Rac1 (a member of the p21 GTPase family) appeared to contribute to HIF-1α regulation, Diebold *et al*. (9) focused on investigating the downstream signalling events in pulmonary artery smooth muscle cells (PASMCs). They found that, downstream from Rac1, the p21-activated kinase-1 (PAK-1) and HIF-1α are linked in pulmonary vascular remodelling. Thereby, active PAK-1 enhanced HIF-1α transcription via NF-κB. Conversely, HIF-1 itself promoted transcriptional upregulation of *Rac1* and *PAK-1*, making PAK-1 and its activator Rac1 new novel HIF-1 targets. Although this positive-feedback loop explains the elevated levels of PAK-1, Rac1, and the HIF-1 target plasminogen activator inhibitor-1 (*PAI-1*) in remodeled pulmonary vessels, this mechanism may work in a modified manner in other tissues. This is indicated in the article of Dimova *et al*. (11), who investigated the regulation of the *PAI-1* gene by peroxide-induced oxidative stress in liver-derived cells. They found that peroxide increased *PAI-1* transcription, and that this was mediated indirectly through FOXO4, which downregulated HIF-1α levels but upregulated CREB levels and subsequently CREB binding to the *PAI-1* promoter. Thus, these findings indicate, although tissue differences may exist, a link between increased *PAI-1* gene expression in response to ROS, and the detected overexpression of PAI-1 in patients with pulmonary hypertension or nonproliferative diabetic retinopathy, in which the decreased matrix degradation due to PAI-1–induced inhibition of plasmin formation may contribute to the observed vessel abnormalities.

Although the regulation of HIF-1α by ROS has been widely addressed, only limited information is available regarding the regulation of HIF-2α by ROS. Thrombin has been recognized as an important stimulus of ROS production by NADPH oxidases, and in the study by Diebold *et al*. (8), they investigated whether thrombin and NADPH oxidases regulate HIF-2α in pulmonary artery smooth muscle cells (PASMCs). They found that activation of the endoplasmic reticulum–localized NADPH oxidase, containing the NOX4 subunit by thrombin or H_2O_2, increased HIF-2α protein levels in PASMCs. This response was due to diminished pVHL binding and was disrupted by treatment with ascorbate. Mutation of specific proline or asparagine residues involved in HIF-2α hydroxylation also abolished these effects, indicating that ROS derived from NOX4 in response to thrombin stabilize and activate HIF-2α by preventing hydroxylation of the N- and C-TAD, thus allowing formation of transcriptionally active HIF-2α.

Interestingly, a number of nutrients of plant origin contain flavonoids, which are considered to be beneficial in inflammation and cancer due to their radical-scavenging properties. Especially quercetin exerts a variety of biologic activities at nontoxic concentrations in organisms, but the molecular details of quercetin effects associated with antithrombotic, antihypertensive, anti-inflammatory, and anticarcinogenic actions (12) are not yet fully elucidated. In their study, Bach *et al.* (2) found that quercetin induces HIF-1α by interfering with the proline hydroxylation-dependent HIF-1α protein destabilization. These effects were independent of the ROS-scavenging activities but required the flavonol structure and the presence of hydroxyl groups at position 3′ and 4′ of the molecule. Further, quercetin induced the cell-cycle inhibitor *p21WAF*, and knocking down HIF-1α disrupted these effects. These results extend our understanding of the mechanisms by which especially quercetin mediates its effects and provide insight into the quercetin-mediated regulation of growth arrest through HIF-1–dependent induction of p21WAF expression.

Whereas the studies of ROS generation in each single cellular compartment, especially under stress conditions, have established quite clearly a set of regulatory pathways determining enzyme function and gene expression, reports concerning the more physiologic effects of ROS as putative communicators between the different compartments are pending. Apparently, conflicting observations concerning ROS-dependent effects may possibly arise from differences in the relative expression levels of interacting molecules, the cell type, the stage of the tumor or disease, or even differences in the experimental design. Further progress will be gained by the use of agents specifically inhibiting/generating ROS in each compartment, so that molecular interactions can be studied in a more-specific manner. Although a completely consistent picture with respect to ROS actions in the intracellular compartments has not been elucidated, and a number of mechanistic details must be clarified, I think that the ROS field is still exciting, and I hope that the contributions of this Forum Issue will be useful for insiders and also will attract new people to the field.

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