SnapShot: Reactive Oxygen Intermediates (ROI)

Cell

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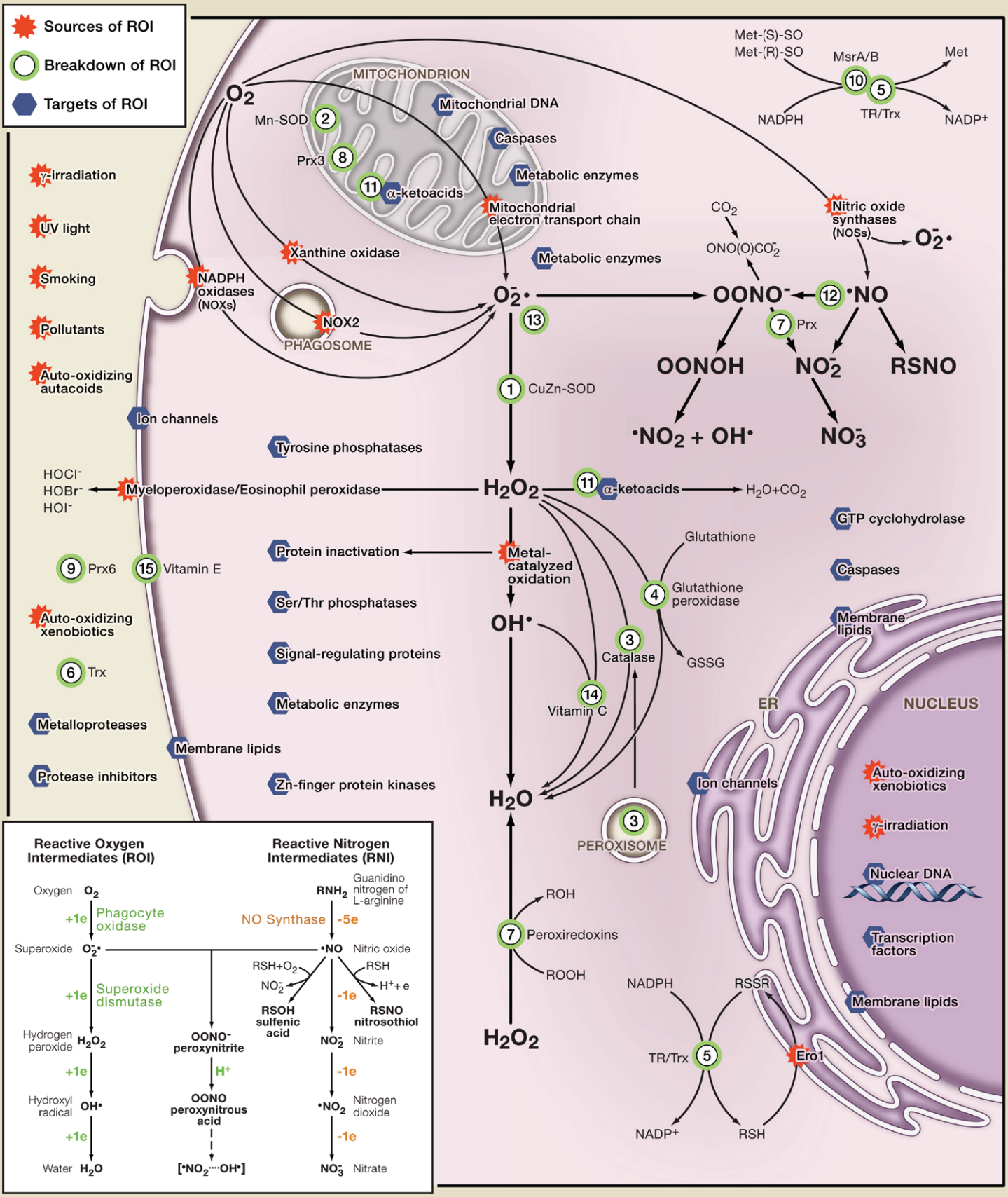

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Reactive oxygen intermediates (ROI) play diverse roles in inflammation, host defense, and homeostasis. For example, ROI produced by neutrophils kill pathogens engulfed by these innate immune cells during the early steps of the inflammatory response to infection. In this SnapShot, we define ROI and related molecules, summarize their reactions, characterize their specificity, and list their sources (red), targets (blue), and catabolic routes (green; also called "anti-oxidant defenses").

Terminology: ROI, ROS, and RNI

Reactive oxygen intermediates (ROI) are successive 1-electron reduction products of O_2 en route to the production of water (see inset). ROI include superoxide anion radical (O_2^- or $O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^\bullet OH$). Reactive oxygen species (ROS) include ROI plus ozone (O_3) and singlet oxygen ($^1O_2$) (whose production by cells is less clear). The terms ROI and ROS are sometimes used interchangeably and may include hypochlorous (HOCl), hypobromous (HOBr), and hypoiodous acids (HOI), which arise by peroxidase-catalyzed oxidation of halides. The reactive nitrogen intermediates (RNI) that most closely influence ROI levels are nitric oxide radical (NO or $^\bullet NO$), nitrogen dioxide radical ($^\bullet NO_2$), nitrite (NO_2^-), and peroxynitrite ($ONOO^-$).

Reactions

Reactions of ROI and RNI are covalent but often reversible. Reversible reactions of ROI and RNI include those of H_2O_2 with sulfhydryls as in cysteine (RSH) to yield disulfides (RSSR'), sulfenic acids (RSOH), or (sometimes reversible) sulfinic acids (RSO_2H); of NO with sulfhydryls to yield S-nitrosothiols (RSNO); and of H_2O_2 with the thioether sulfur in methionine to yield methionine sulfoxide. Irreversible reactions include hydroxylations, carbonylations (particularly on peptidyl arginine, lysine, proline, and threonine residues), halogenations of amines, nitrations, formation of sulfonic acids (RSO_3H), and destruction of iron-sulfur clusters.

Reactions of ROI and RNI with macromolecules involve a limited range of specific atoms and only some atoms of that type, depending on their immediate environment. For example, ROI react with sulfur, but usually only in methionine or cysteine. Peptidyl cysteine sulfhydryls become more reactive when present as the thiolate under the influence of positively charged neighboring side chains. This is a characteristic of active-site cysteines such as those found in tyrosine phosphatases. Reactivity with a small number of specific atoms, each found in a large number of molecules, has been termed "atomic specificity." This physiologically complements "molecular specificity," in which a given signaling molecule reacts with a much smaller number of other molecules, initially based on the affinity of their noncovalent interactions. At high levels, ROI are toxic, as with any form of signaling that is inappropriate in time, place, level, or duration. A major means by which host and pathogen compete is to impose maladaptive signaling on each other; production of high levels of ROI and RNI conforms to this principle. The term "anti-oxidant defenses" emphasizes the deleterious effects of high levels of ROI, but enzymes that dispose of ROI are also essential regulators of homeostatic signaling. ROI may act chiefly near their site of production (e.g., when produced by NAPDH oxidases that are coupled with transmembrane receptors) but sometimes act more widely in the cell (e.g., when produced by mitochondria). ROI coordinate other signaling pathways associated with a cell's metabolic state through their dependence on oxygenation, electron transport, the activity of metabolic enzymes, redox state, and levels of α-ketoacids and of NADPH and NADH.

Major Sources of ROI

The mitochondrial electron transport chain is estimated to leak about 1%–2% of its electrons as O_2^-. Hypoxia increases production of ROI by deregulating the mitochondrial electron transport chain. NO and $OONO^-$ promote generation of ROI by mitochondria. This action of NO is also more pronounced in hypoxia. Elevated intracellular Ca^{2+} is another stimulus. Regulators of ROI production include $pp60^{shc}$ and mTOR. Another major source of ROI, especially when there is an excess of unfolded proteins, is the flavoenzyme Ero1 in the endoplasmic reticulum, which is also sensitive to elevated intracellular Ca^{2+}. The third major source of ROI is production by seven NADPH oxidases (NOXs), including five NOXs and two dual oxidase NOX variants. NOXs are activated in response to stimulation of associated receptors, such as those for insulin, platelet-derived growth factor, nerve growth factor, fibroblast growth factor, tumor necrosis factor (TNF), the growth factor GM-CSF, and angiotensin. NOX2 in phagocytes is activated by engagement of receptors for the antibody Fc chain (Fcγ) or for complement during phagocytosis of opsonized particles. NOX2 in neutrophils is also activated by engagement of receptors for integrins simultaneously with engagement of receptors for soluble agonists such as TNF, formyl peptides, complement C5a, the growth factors G-CSF and GM-CSF, or macrophage inflammatory protein. NOXs can be regulated by phosphorylation of p47 or TKS5, by flavin-loading of gp91, by the GTPase activity of Rac1 or Rac2, and by assembly of their components (gp91; p22; p47 or TKS5; p40 or NoxO1; p67 or NoxA1; and Rac1 or Rac2). Nitric oxide synthases (NOSs) can produce O_2^- when "uncoupled" by hypoxia or loss or oxidation of their cofactor, tetrahydrobiopterin (BH_4). Metal-catalyzed oxidations are triggered by an increase in free metals, such as iron or copper, or metals inhaled in microparticulate pollutants. Such metal-catalyzed oxidations include the Haber-Weiss reaction, the metal ion [eg ferric]-catalyzed generation of $^\bullet OH$ from O_2^- and H_2O_2, and the Fenton reaction, the metal ion [eg ferrous]-catalyzed generation of $^\bullet OH$ from H_2O_2. Xanthine oxidase produces O_2^- from xanthine or hypoxanthine and is a prominent source of ROI during ischemia-reperfusion, when sulfhydryl oxidation converts xanthine dehydrogenase into xanthine oxidase. The myeloperoxidase enzyme of neutrophils and monocytes and eosinophil peroxidase produce hypohalous acids during phagocytosis or degranulation. Some autacoids (such as dopamine) undergo auto-oxidation, producing ROI. Some xenobiotics produce ROI when they promote metal-catalyzed oxidation (such as bleomycin) or undergo auto-oxidation (such as adriamycin). This can be central to their mechanism of action or toxicity. γ-irradiation generates $^\bullet OH$. UV light can generate $^1O_2$ in biological samples and induces synthesis of nitric oxide synthase 2 in skin. Smoke and other air pollutants contain ROS and organic radicals.

Prominent Targets of ROI

Tyrosine phosphatases rely on active-site cysteine residues whose sulfhydryl thiolate is subject to reversible, oxidative inactivation. The reversible oxidative inactivation of phosphatases augments the apparent activity of tyrosine kinases. Similarly, many cysteine-dependent serine/threonine phosphatases are subject to reversible oxidative inactivation, so that ROI augment the activation of the kinases MAPK, ERK, and JNK and their associated signaling pathways. ROI may also reversibly oxidize cysteines that coordinate Zn^{2+} in Zn^{2+}-finger proteins, including kinases, leading, for example, to activation of protein kinase C. Metalloproteases can be activated by oxidation of cysteine residues that help to maintain the inactive state by coordinating Zn^{2+}. Caspases can be transiently inhibited through reversible inactivation of the active-site cysteine, blocking apoptosis and the production of IL-1β, IL-18, and IL-33. Reversible cysteine oxidation can affect a number of signal-regulating binding proteins, such as 14-3-3 and heat shock proteins, potentially affecting the regulation of numerous other signaling molecules. Transcription factors are a major target, both through oxidative inactivation (e.g., SoxR) or activation that can be direct (e.g., OxyR) or indirect (e.g., Nrf2 through oxidation of KEAP). Activation of the transcription factors AP-1 and NF-κB by ROI may be direct or indirect. Certain enzymes of intermediary metabolism are also inhibited by ROI, including α-ketoglutarate dehydrogenase and pyruvate dehydrogenase. A major impact of H_2O_2 on cell metabolism is through oxidative decarboxylation of α-ketoacids, such as pyruvate, α-ketoglutarate, and oxaloacetate. Diverse proteins are subject to irreversible inactivation through metal-catalyzed oxidation and carbonylation of arginine, lysine, proline, and threonine residues. Oxidative inactivation of GTP cyclohydrolase, the rate-limiting enzyme for formation of BH_4, can divert NOSs to produce O_2^- rather than NO. Of major importance in inflammation, the protease inhibitors that play the greatest role in restraining tissue damage by neutrophil proteases are subject to oxidative inactivation by neutrophil-derived ROI. These include secretory leukocyte protease inhibitor (SLPI), α2-macroglobulin, and α1-antitrypsin. Some ion channels can be activated by ROI, such as transient receptor potential 1 (TRPA I). Nuclear and mitochondrial DNA is a target for mutagenesis by ROI. ROI actions on diverse lipids can have widespread consequences, for example, through formation of isoprostanes or oxidation of arachidonyl-palmitoyl-phosphatidylcholine to generate an agonist for Toll-like receptor 4 (TLR4).

(*continued on following page*)

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Breakdown of ROI

The numbered pathways in green represent different routes for breakdown of ROI. (1) CuZn-superoxide dismutase (SOD) in the cytosol converts O_2^- to H_2O_2, thereby decreasing formation of $ONOO^-$. (2) Mn-SOD in the mitochondria of eukaryotic cells and FeSOD and NiSOD in prokaryotes catalyze the same reaction. (3) Catalase in eukaryotic peroxisomes and in prokaryotes acts on high levels of H_2O_2 forming H_2O and O_2. At low H_2O_2 levels, catalase acts as a peroxidase, oxidizing other substrates. (4) The glutathione (GSH) redox cycle is driven by NADPH and includes the abundant cysteinyl tripeptide GSH, GSH reductase, glutaredoxins, and GSH peroxidase. This cycle reduces disulfide bonds, donates an electron to some peroxiredoxins (Prx) or ascorbate (vitamin C), reverses protein S-nitroso bonds via transient glutathionylation of cysteinyl sulfhydryls, and detoxifies xenobiotics. (5) The thioredoxin (Trx) redox cycle is driven by NADPH and includes the oxidoreductase Trx, Trx reductase (TR), and Trx peroxidase (TPO). The Trx redox cycle reduces protein disulfide bonds and donates electrons to peroxiredoxins, methionine sulfoxide reductases, and ribonucleotide reductase. Trx binds and regulates certain signaling proteins. (6) Secreted Trx can reduce protein disulfide bonds and has chemotactic and cytokine-like properties. (7–9) Peroxiredoxins (Prx) 1–6 reduce H_2O_2, organic peroxides, and $ONOO^-$. (7) Prx 1, 2, 4, and 5 are cytosolic. (8) Prx3 is in the mitochondrial matrix. (9) Prx6 is secreted. (10) Distinct methionine sulfoxide reductases catalyze the stereospecific reduction of methionine-(S)-sulfoxide (MsrA) or methionine-(R)-sulfoxide (MsrB) to methionine. This is a significant route for consumption of H_2O_2 at the expense of NADPH, with proteins that present peptidyl methionines acting as platforms (not shown). (11) α-ketoacids (e.g., pyruvate, oxaloacetate, α-ketoglutarate) react rapidly, nonenzymatically, and stoichiometrically to consume H_2O_2 (and vice versa) through oxidative decarboxylation. (12) NO diverts O_2^- from forming less reactive H_2O_2 and instead induces the formation of $OONO^-$ and its more reactive products, $^{\bullet}OH$ and $^{\bullet}NO_2$. (13) O_2^- diverts less reactive NO to form $OONO^-$ and its more reactive products, $^{\bullet}OH$ and $^{\bullet}NO_2$. (14) Vitamin C reduces H_2O_2 and scavenges $^{\bullet}OH$. (15) Tocopherols (vitamin E) may react with lipid radicals induced by ROS.

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