CELL SIGNALING

H_2O_2, a Necessary Evil for Cell Signaling

Once considered lethal to cells, reactive oxygen species are now known to be involved in redox signaling pathways that may contribute to normal cell function as well as disease progression.

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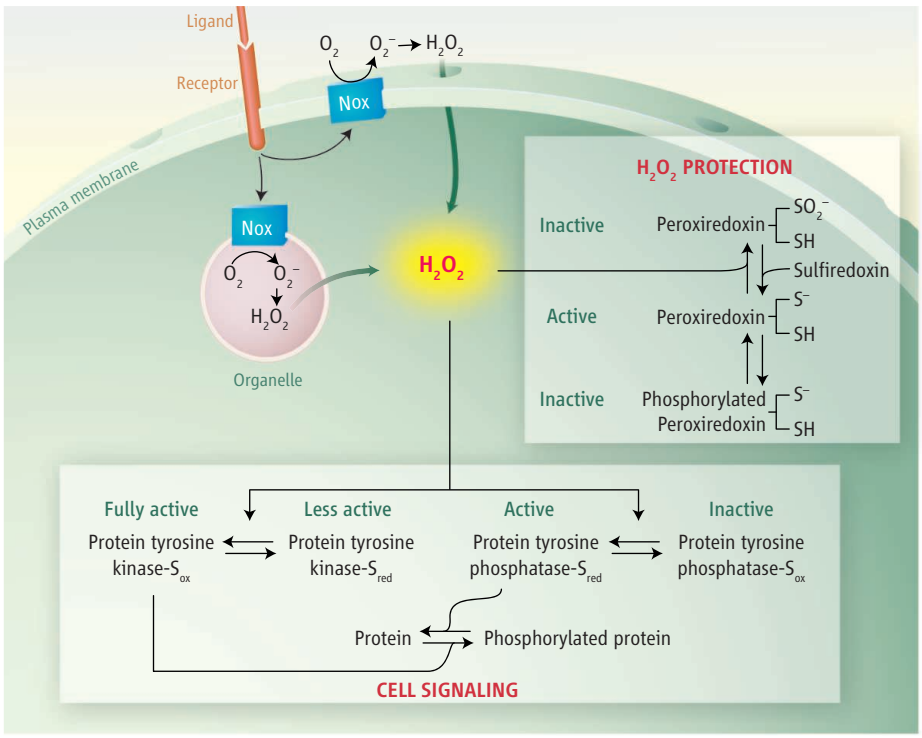
For many years, hydrogen peroxide (H_2O_2) was viewed as the inevitable but unwanted by-product of an aerobic existence. Given the damage inflicted by H_2O_2, it was assumed that the faster the elimination of this toxic waste, the better for the cell. However, as highlighted in recent forums (*1*, *2*), we now know that mammalian cells produce H_2O_2 to mediate diverse physiological responses such as cell proliferation, differentiation, and migration (*3*, *4*). This has led to implications of cellular "redox" signaling in regulating normal processes and disease progression, including angiogenesis, oxidative stress and aging, and cancer. This changing view of H_2O_2 has partly evolved from a clearer understanding of redox chemistry as it affects biology—that is, cellular signaling that is linked to reductive-oxidative–based mechanisms. As the components and mechanisms involved in performing cellular redox chemistry become better defined, new areas of research are emerging as to how the cells spatially and temporally channel H_2O_2 into specific signaling pathways to achieve desired cellular outcomes.

H_2O_2 production has been studied most extensively in neutrophils. These immune cells defend a host against infections by engulfing and killing foreign microorganisms. The system relies on Nox [the NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase complex], which generates millimolar quantities of H_2O_2 within the safe confines of an organelle [phagosome (see the figure)] for the purpose of microbial killing. In the classical phagocyte paradigm, stimulation of neutrophils by invading microoganisms leads to assembly at the plasma membrane of an active Nox complex, which comprises a catalytic subunit—the integral membrane protein gp91 Phox—and regulatory proteins including the small guanosine triphosphatase Rac. This complex releases the reactive oxygen species superoxide (the free radical anion O_2^{-}) into the phagosome, and superoxide dismutation yields another reactive oxygen species, H_2O_2.

We do know that in nonphagocytic cells, H_2O_2 affects numerous intracellular signaling pathways. Nonphagocytic cells express gp91 Phox and its homologs (*5*), and these proteins are the major source of H_2O_2 in cells stimulated with various growth factors and cytokines including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, tumor necrosis factor–α (TNFα), and interleukin-1 (IL-1) (*3*, *4*). However, the coupling of receptor activation to Nox activation in nonphagocytic cells still remains poorly understood.



H_2O_2 production, protection, and signaling actions. Activation of various cell surface receptors activates Nox situated either in the plasma membrane or in the membrane of organelles such as endosomes to produce H_2O_2. To function as an intracellular signaling molecule, H_2O_2 must be imported into the cytosol. Cytosolic H_2O_2 enhances protein tyrosine phosphorylation by inactivating protein tyrosine phosphatases while activating protein tyrosine kinases. Transient protection of the H_2O_2 signal from abundant cytosolic peroxiredoxin appears to result from the reversible inactivation of these enzymes through either hyperoxidation or phosphorylation.

We are also trying to understand the mechanisms by which H_2O_2 can modify the activity of key signaling proteins. Biological redox reactions catalyzed by H_2O_2 typically involve the oxidation of cysteine residues on proteins, which may affect protein function. Phosphorylation of tyrosine residues in proteins is governed by the opposing activities of protein tyrosine phosphatases and protein tyrosine kinases. The protein tyrosine phosphatase family features a common Cys-X-X-X-X-X-Arg active-site motif (where X = any amino acid). As a result of the invariant arginine, the conserved catalytic cysteine possesses a low pK_a (where K_a is the acid dissociation constant) and exists as a thiolate anion with enhanced susceptibility to oxidation by H_2O_2. Oxidation of the essential cysteine abolishes phosphatase activity and can be reversed by cellular thiols. Reversible inactivation of several different protein tyrosine phosphatases has been demonstrated in relevant cell types stimulated with PDGF, EGF, insulin, extracellular matrix molecules, and B cell receptor ligands (*3*, *6*). Oxidative inactivation of these phosphatases and increased tyrosine phosphorylation of target proteins were found to be dependent on H_2O_2 production. Moreover, in TNF-α–stimulated cells, the resulting H_2O_2 that is generated inactivates mitogen-activated protein kinase phosphatases. This in turn results in sustained activation of c-Jun N-terminal kinase, a subfamily of the mitogen-activated protein kinases that elicits specific cellular responses.

H_2O_2 also appears to promote tyrosine phosphorylation by activating protein tyrosine kinases. For example, upon cell attachment to extracellular matrix and associated generation of H_2O_2, the tyrosine kinase Src becomes oxidized at two cysteine residues and thus becomes acti-

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Published by AAAS

vated (*7*). Moreover, antioxidant treatment of cells that express an oncogenic form of Src (v-Src), or mutation of the oxidation-sensitive cysteine residues of v-Src, reduces the potency of v-Src to transform cells. This redox-dependent activation of Src occurs alongside dephosphorylation of a carboxyl-terminal tyrosine, a modification that is needed to activate Src.

For H_2O_2 to serve as a signal—through modification of signaling proteins—its concentration must increase rapidly above a certain threshold. How can this occur in the presence of antioxidant enzymes such as catalase, glutathione perioxidase, and peroxiredoxin? Whereas catalase is confined to the peroxisome, several peroxiredoxin isoforms are abundant in the cytosol. Therefore, H_2O_2 must be protected from destruction by peroxiredoxin in selected contexts. Indeed, multiple protective mechanisms of this type are being uncovered. During catalysis of H_2O_2 reduction, the active-site residue, Cys-SH, of peroxiredoxin occasionally reacts with two molecules of H_2O_2, and thus becomes hyperoxidized to Cys-SOOH. Consequently, peroxiredoxins are inactivated (*8*). This inactivation, which can be reversed by sulfiredoxin, an adenosine triphosphate–dependent enzyme, may represent a built-in mechanism to prevent damping of the H_2O_2 signal. Prokaryotes do not express sulfiredoxin and their peroxiredoxins are resistant to hyperoxidation. Thus, this regulatory mode appears unique to eukaryotes. Peroxiredoxins are also reversibly inactivated upon phosphorylation by cyclin B–dependent kinase during mitosis (*9*).

Given the toxicity of H_2O_2, spatial and temporal regulatory strategies must exist to ensure that Nox activation occurs only where needed and that the H_2O_2 signal is terminated in a timely fashion. Recent work on cells stimulated with TNF-α. suggests that Nox proteins are assembled in specific subcellular compartments within membranes such as lipid rafts (*10*). Localized Nox assembly also occurs at focal complexes, points of contact between a moving cell and the extracellular matrix, in response to migratory stimuli (*11*). The relevant oxidation targets that are presumably enriched in these microenvironments remain to be identified.

Despite the increasingly sophisticated molecular descriptions of H_2O_2 action, disturbingly little is understood about how H_2O_2 is actually delivered to the cytosol. The classical neutrophil studies demonstrate that Nox releases H_2O_2 into the phagosome, which is topologically equivalent to the extracellular space. How, then, does H_2O_2 modulate intracellular signaling? In one scenario, Nox situated at the plasma membrane releases H_2O_2 into the extracellular space as an autocrine factor to be imported into the cell. Alternatively, Nox proteins assembled at organelle membranes discharge H_2O_2 into the lumenal space. For example, binding of IL-1 to its receptor in the plasma membrane triggers Rac-mediated Nox association with the IL-1 receptor and endocytosis (internalization) of the receptor complex (*12*). This results in superoxide production and conversion into H_2O_2 in the lumen of the endosome. In addition, Nox isoforms and their regulatory subunits have been detected in other cell organelles including the endoplasmic reticulum and nucleus.

Regardless of whether the Nox complex is activated at the cell surface or within an organelle, the resultant H_2O_2 must traverse the lipid bilayer to access the cytosol, where most if not all of its target proteins exist. Although H_2O_2 is believed to diffuse freely across membranes, recent studies indicate that some membranes are poorly permeable to H_2O_2. Instead, H_2O_2 transport might be regulated by changes in membrane lipid composition or by aquaporins (*13*), which are diffusion-facilitating channel proteins for noncharged solutes such as water.

The current picture of H_2O_2-based redox regulation of signaling processes is rapidly expanding beyond those issues focused on here. The development of a sensitive and specific probe for H_2O_2 that allows quantitative and dynamic assessment in live cells, conspicuously lacking in studies to date, will be a great boon for the study of this misunderstood and maligned molecule.

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14. S.G.R.'s research is supported by Grant FPR0502-470 from the Korean Ministry of Science and Technology.

10.1126/science.1130481

MATERIALS SCIENCE

Seeking Room-Temperature Ferromagnetic Semiconductors

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Microelectronic circuits that retain their logic state when the power is off would permit entirely new kinds of computers. Ferromagnetic semiconductors might make this technology possible.

Nonvolatile digital circuits that retain their logic states even when their power sources are rapidly switched on and off would make possible a new type of computer. Although appearing to operate normally, these devices would actually be turned off most of the time, potentially changing the way we use digital devices. Such devices would allow, for example, year-long operation of mobile computers, an enormous number of tiny computers embedded all around us to help our daily lives, and ultra-high-density integrated circuits free from heat generation problems. To make this dream a reality, nonvolatile transistors are needed, but unfortunately this technology is nonexistent. Because transistors are composed of semiconductor materials, the ideal way to make nonvolatile transistors would be to use semiconductor materials that are intrinsically nonvolatile.

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Among several physical phenomena that produce nonvolatility, the most enticing is that of ferromagnetic hysteresis. In this effect, the material retains its magnetic state until reversed by a suitable magnetic field. Ferromagnetism has been verified to offer high-speed, unlimited magnetization reversal, so it is perfect for transistor applications. However, the ferromagnetic materials used in digital devices such as hard disks and magnetic random access memory chips—iron, cobalt, and nickel and their alloys—are not semiconductor materials. Hence, there is a continuing search for semiconductor materials that display ferromagnetic properties.

By replacing some of the positive ions of the parent nonmagnetic semiconductors by magnetic ions, one can make ferromagnetic semiconductors such as (In,Mn)As and (Ga,Mn)As (*1*). But their ferromagnetic Curie temperatures (T_c)—the temperature at which the ferromagnetism disappears—are 61 K (*2*) and 173 K (*3*), respectively, much lower than room temperature. In 2000, Dietl *et al.* (*4*) used a simple theory to estimate the T_c of