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Redox signaling: hydrogen peroxide as intracellular messenger

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Abbreviations: TNF, tumor necrosis factor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; ROS, reactive oxygen species; NO, nitric oxide; PTPIB, protein tyrosine phosphatase 1B

Abstract

Although superoxide anions ($O_2^{\cdot-}$) and H_2O_2 are generally considered to be toxic by-products of respiration, recent evidence suggests that the production of these reactive oxygen species (ROS) might be an integral component of membrane receptor signaling. In mammalian cells, a variety of extracellular stimuli have recently been shown to induce a transient increase in the intracellular concentration of ROS, and specific inhibition of the ROS generation resulted in a complete blockage of stimulant-dependent signaling. In the next few years, therefore, a flurry of research activity is expected in relation to the elucidation of ROS production in response to receptor stimulation, identification of ROS target molecules, and investigation of ROS elimination. The goal of this report is to review our current knowledge of ROS-regulated signal trans-duction and propose future directions.

Keywords: redox, hydrogen peroxide, ROS, signaling

Introduction

The process that transduces the extracellular messages carried by first messengers such as hormones, growth factors, cytokines, and neurotransmitters across plasma membranes into the intracellular components is called signal transduction or cell signaling. An important feature of the signal transduction is that the first messenger molecules need not enter the cell and their biological effects are mediated inside the cell by second messenger molecules such as cAMP, cGMP, inositol 1,4,5-trisphosphate (Ins 1,4,5-P3), nitric oxide, and phosphatidylinositol 1,3,4,5-tetrakisphosphate (PtdIns 1,3,4,5-P4). The second messenger generation process involves various cellular components like specific receptors, transducers, adaptor proteins, protein kinases, and protein phosphatases, and eventually lead to the induction of physiological responses such as gene expression, cell proliferation, secretion, cell motility, and neurotransmission.

Recently, reactive oxygen species (ROS) gained attention as important second messengers. For example, hydrogen peroxide (H_2O_2) mimics the stimulatory effects of insulin on glucose transport and lipid synthesis in adipocytes (May and De Haen, 1979). The production of ROS has been detected in a variety of cells stimulated with cytokines such as transforming growth factor-β1 (TGF-β1) (Ohba *et al*., 1994; Thannickal and Fanburg, 1995), interleukin-1 (Meier *et al*.,1989), and tumor necrosis factor-α (TNF-α) (Meier *et al*.,1989; Lo and Cruz, 1995), with peptide growth factors such as platelet-derived growth factor (PDGF) (Krieger-Brauer and Kather, 1995; Sundaresan *et al*., 1995), epidermal growth factor (EGF) (Bae *et al*., 1997), and basic fibroblast growth factor (bFGF) (Krieger-Brauer and Kather, 1995; Lo and Cruz, 1995), with agonists of receptors with seven transmembrane spans such as angiotensin II (Griendling *et al*., 1994), thrombin (Chen *et al*., 1995) and lysophosphatidic acid (Chen *et al*., 1995) or with phorbol ester (Robertson *et al*., 1990) and phosphatidic acid (Lee *et al*., 1998). The term ROS encompasses many species including singlet oxygen, the superoxide anion radical ($O_2^{\cdot-}$), H_2O_2, lipid peroxides, nitric oxide (NO), peroxynitrite ($ONOO^-$), the thiyl peroxyl radical ($RSOO^{\cdot}$), the ferryl radical (FeO^{2+}) and the hydroxyl radical ($OH^{\cdot}$) (Moncada *et al*., 1991; Stadtman and Oliver, 1991; Huie and Padmaja, 1993; Yim *et al*., 1994).

Although the chemical nature of ROS generated in response to the activation of various receptors has not been well characterized, H_2O_2 was shown to be a major component of ROS in cells activated by TGF-β1, PDGF, EGF, or angiotensin II (Ohba *et al*., 1994; Sundaresan *et al*., 1995; Bae *et al*., 1997). The generation of ROS in response to various external stimuli has been related to the activation of transcription factors such as NF-κB (Schreck *et al*., 1991) and AP-1 (Pahl and Baeuerle, 1994; Lo and Cruz, 1995), mitogen-activated protein (MAP) kinases (Chen *et al*., 1995; Sundaresan *et al*., 1995), phospholipase A_2 (Zor *et al*., 1993), protein kinase C (Konishi *et al*., 1997), and phospholipase D (Natarajan *et al*., 1993; Min *et al*., 1998), to increasing cytosolic calcium (Suzuki *et al*., 1997), to the triggering of apoptosis (Jacobson, 1996), to the inhibition of protein tyrosine phosphatases (PTPase) (Hecht and Zick, 1992; Sullivan

et al., 1994; Lee *et al*., 1998), and to the alteration of ion transport mechanisms (Kourie, 1998). H_2O_2 is a small, diffusible, and ubiquitous molecule that can be synthesized, as well as destroyed, rapidly in response to external stimuli. As such it fulfills the important prerequisites for an intracellular messengers.

Even in the absence of extracellular stimulation, super-oxide anions ($O_2^{\cdot -}$) is constantly produced by metabolic reactions in all aerobic organisms. The $O_2^{\cdot -}$ is then spontaneously or enzymatically dismutated to H_2O_2. In addition, cells are exposed to irradiations (x-rays, gamma-rays, ultraviolet light), inflammatory systems, metal-catalyzed oxidation systems, environmental pollutants inevitably produce ROS (Stadtman and Berlett, 1998). Whereas any one of the ROS is able to damage cellular components, hydroxyl radicals (OH), causes damage indiscriminately. And there is reason to believe that the most important mechanism of OH generation inside cells is from H_2O_2 via Fenton reaction [H_2O_2 + Fe(II) or Cu(I) and OH^- + OH + Fe(III) or Cu(II)] (Stadtman and Berlett, 1998). Cellular electron donors such as NADH, NADPH, ascorbate, various thiol compounds are able to regenerate Fe(II) or Cu(I). For these reasons,$O_2^{\cdot -}$ and H_2O_2 have been thought of as the unwanted and toxic byproducts of living in an aerobic environment. Though the cell had clearly evolved mutiple defences for their elimination, their relentless production coupled with their damaging nature has led to the widely held belief that these molecules serve only a harmful function.

However, the notion that molecules like $O_2^{\cdot -}$ and H_2O_2 could function in signal transduction in mammalian cells is not without precedent. Indeed, a wealth of information suggests that these molecules serve as signaling molecules in both bacteria and plants. In bacteria, redox regulation of transcription occurs, with a different set of genes stimulated by $O_2^{\cdot -}$ and H_2O_2 (Hidalgo *et al*., 1997; Rosner and Storz, 1997; Zheng *et al*., 1998). Similarly in the plant response to pathogen, there appears to be a clear role for H_2O_2 as a signaling molecule (Levine *et al*., 1994; Shirasu *et al*., 1996). Salicylic acid binds to and inactivates tobacco catalase, leading to a rise in H_2O_2 concentration and the activation of gene transcription (Chen *et al*., 1993). In mammalian cells, the physiological role for $O_2^{\cdot -}$ and H_2O_2 is less characterized than that of another ROS, namely nitric oxide (NO).

Analysis of the role of NO suggests it fuctions in two discrete fashions. Production of NO by macrophages and other immune cells results in the high level production of NO, consistent with its role in host defense. In contrast, the nitric oxide synthetase in nonphagocytic cells produce 2-3 orders of magnitude less NO when activated. Product at this level, NO is widely believed to function in cell signaling (Finkel, 1998). This dichotomy between defence function and signal transduction is likely to be preserved for $O_2^{\cdot -}$ and H_2O_2. $O_2^{\cdot -}$ and H_2O_2 are also produced in large amounts by phagocytic cells. In contrast, other cell types appear to produce significantly lower amounts of these molecules. Emerging evidence suggests that this minimal oxidative burst appears to serve an important role in signal transduction (Finkel, 1998).

Since the introduction of the second messenger concept as the result of the pioneering work on cAMP by Earl W. Jr. Sutherland (Recipient of the 1970 Nobel Prize in Physiology and Medicine), all branch of life sciences including medicine have gained tremendous insight by studying how receptor occupation elicits the production of a second messenger, what kinds of molecules are targeted by the produced messenger, and how the messenger molecules are eliminated after the completion of their mission. For example, related to cAMP and cGMP, studies on the coupling mechanism of receptors to adenylate and guanylate cyclases, protein phosphorylation events initiated by the activation of cyclic nucleotide-dependent kinases, removal of cAMP and cGMP by phosphodiesterases have been the center pieces of Biochemistry, Pharmacology, Endocrinology, and Immunology for many years. These studies led to the discovery of G-proteins by Alfred G. Gilman and Martin Rodbell (Recipients of the 1994 Nobel laureates in Physiology and Medicine) and to the discovery by Edmond H. Fischer and Edwin G. Krebs (Recipients of the 1992 Nobel laureates in Physiology and Medicine) of fundamental principles concerning reversible protein phosphorylation as a biological regulatory mechanism. These studies provided not only new principles of science but many useful sites where useful therapeutic agents can be designed for intervention. Viagra, developed as an inhibitor of cGMP phosphodiesterase is an example. It is also significant to note that the 1998 Nobel Prize in Physiology and Medicine was given, in recognition of the discoveries concernig nitric oxide as a signaling molecule, to Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad.

At the present time, very little is known about 1) the mechanism by which H_2O_2 is generated in response to receptor stimulation in nonphagocytic cells, 2) the molecules on which H_2O_2 acts to propagate the signal, and 3) the controlled pathway by which H_2O_2 is timely removed. Studies on these three subjects, production, target, and elimination, are expected to be important area in biochemistry.

Production of H_2O_2 in response to receptor stimulation in nonphagocytic cells

The mechanism of the receptor-mediated generation of ROS has been studied extensively in phagocytic cells, in which $O_2^{\cdot -}$ (and thus H_2O_2) is produced via the reduction of O_2 by a complicated enzyme system named NADPH

oxidase (Thelen *et al*., 1993). The NADPH oxidase consists of at least four polypetide components, two cytosolic components (p47- and p67-phox) and two transmembrane flavocytochrome b components (gp91- and p22-phox). The activation of NADPH oxidase involves a specific protein-protein interaction (via SH3-prolin-rich domains) and phosphorylation by various protein kinases At least one additional protein, the small GTP-binding protein Rac (either Rac 1 or Rac 2), is needed for NADPH oxidase activation. In contrast, the mechanism of H_2O_2 generation in nonphagocytic cells remains unclear.

Neither immunoblot nor Northernblot analysis using molecular probes derived from the NADPH oxidase components of phagocytes detected similar components in nonphagocytic cells. However, the production of H_2O_2 in fibroblasts stimulated by PDGF, EGF, Il-1, or TNF-α appears to be mediated by a Rac-sensitive system (Sundaresan *et al*., 1996). Furthermore, treatment with diphenyleneiodonium, a pharmacological inhibitor of the flavoprotein component of the neutrophil NADPH oxidase, appears to significantly affect ROS production in nonphagocytic cells stimulated by first messengers or expression of activated rac (Griendling *et al*., 1994; Ohba *et al*., 1994). In addition, inhibiting the expression of one of the ubiquitously expressed components, p22-phox, was recently shown to inhibit the ability of angiotensin II to stimulate H_2O_2 production in vascular smooth muscle cells (Ushio-Fukai *et al*., 1996). Together with the fact that overproduction of Rac in fibroblasts enhances H_2O_2 production, these results suggest that the system responsible for H_2O_2 ($O_2^{\cdot-}$) production in nonphagocytes is structurally and genetically distinct but functionally similar to the NADPH oxidase system of phagocytic cells.

Evidence suggests that the receptor-triggered generation of H_2O_2 in nonphagocytic cells occurs independently of the mitochondrial respiratory chain and xanthine oxidase system (Meier *et al*.,1989). However, a variety of cellular enzymes including cyclooxygenases and lypoxygenases are also potential agonist-activated H_2O_2 generating system.

Because uncontrolled generation of H_2O_2 is toxic to cells, basal actvity of the putative nonphagocytic NADPH oxidase is expected to be very low. As with other second sessenger-generating systems (e.g. adenylate cyclase and phospholipase C) widely used in receptor signaling, various protein and lipid kinases (phosphatases) and G proteins are likely to be involved in the modulation of the nonphagocytic NADPH oxidase. Additionally, these nonphagocytic components are expected to be subject to control by other second messenger-generating cascades. Considering that the nonphagocytic NADPH oxidase must maintain near zero basal activity and be activated in response to different (cytokine, growth factor, G protein-coupled) receptors, new modes of signal propagation are expected to be uncovered during the study.

In an effort to understand the H_2O_2 production mechanism, the dependence of H_2O_2 production on the intrinsic tyrosine kinase activity of the EGF receptor and the autophosphorylation sites located in its COOH-terminal tail was investigated (Bae *et al*., 1997). EGF failed to induce H_2O_2 generation in cells expressing a kinase-inactive EGF receptor. However, normal H_2O_2 generation was observed in cells expressing a mutant receptor from which the 126 COOH-terminal amino acids had been deleted to remove four (out of the total of five) autophosphorylation sites. These relts suggest that EGF-induced H_2O_2 formation requires the kinase activity, but probably not the autophosphorylation sites, of the EGF receptor.

Target molecules on which H_2O_2 acts to propagate the signal

H_2O_2 is generated in all aerobic organisms as the result of normal cellular metabolism. Since H_2O_2 is readily converted to hydroxyl radicals via the Fenton reaction, it is considered a cytotoxic agent that causes damage to many cellular components. Oxidative damage has been implicated in numerous disorders and the general process of aging. Accordingly, the target for ROS action has been studied mainly in relation to these disorders, leading to the identification of irreversibly damaged cellular components. The second messenger role of H_2O_2 requires reversible modification by or binding of H_2O_2. However, to date no serious efforts to identify such targets can be found in literature.

Unlike cAMP and Ins 1,4,5-P_3, H_2O_2 is too simple structurally to be recognized specifically by proteins. Thus, it is unlikely that reversible binding of H_2O_2 to proteins is a means of propagating a specific signal. On the other hand,H_2O_2 is a mild oxidant that can oxidize specific protein sulfhydryl groups, producing proteins with cysteine sulfenic acid (CysS-OH) or disulfide residues, both of which can easily be reduced back to Cys-SH by various cellular reductants. Very few proteins are expected to have a Cys-SH that is susceptible to oxidation by H_2O_2 in cells because oxidation requires that the target Cys-SH have a pKa below 7.0, whereas the pKa values of most protein Cys-SH residues are higher than 8.0. Proteins like thioredoxin (Holmgren, 1989) protein disulfide isomerase (Freedman *et al*., 1994), protein tyrosine phosphatases (PTPases) (Lohs *et al*., 1997; Lee *et al*., 1998), cysteine proteases (Caspases) (Ischiropoulos, 1998), and peroxiredoxins (Choi *et al*., 1998), which are known to contain an essential Cys-SH with a low pKa at their active sites, are potential candidates for reversible oxidation by intracellularly generated H_2O_2. Protein methionine residues are also sensitive to oxidation by H_2O_2 and easily converted to methionine sulfoxide, and cells are known to contain protein methionine sulfoxide reductase that reduces the oxidized methionine using

NADPH (Levine *et al*., 1996). However, unlike cysteine residues that have been defined for the structural and catalytic roles in many proteins, no known catalytic and regulatory functions have been associated with methionine.

Several serine/threonine kinases such as cAMP-dependent kinase (PKA), cGMP-dependent kinase (PKG), Akt kinase, S6 kinase, and protein kinase C (PKC) all contain a Cys-SH residue within their active site domain (Hanks and Quinn, 1991). Interestingly, these Cys-SH residues are not required for activity but they are fully conserved among these kinases. One can hypothesize that the oxidation of these conserved Cys residues, although their pKa values are not known, is related to the observation that the activity of these kinases are altered in cells treated with H_2O_2. Interaction with regulatory subunits (or molecules) may be modulated by the reversible oxidation of this Cys-SH.

The signal transduction roles of H_2O_2 generated in response to peptide growth factor were studied with PDGF and EGF (Sundaresan *et al*., 1995; Bae *et al*., 1997). Stimulation of rat vascular smooth muscle cells (VSMCs) by PDGF transiently increased the intracellular concentration of H_2O_2, and this increase could be blunted by increasing the intracellular concentration of catalase (Sundaresan *et al*., 1995). The responses of VSMCs to PDGF, which includes tyrosine phosphorylation, MAP kinase activation, DNA synthesis, and chemotaxis, was inhibited when the PDGF-stimulated rise in H_2O_2 concentration was blocked. In another experiment, the elimination of H_2O_2 by catalase in A431 human epidermoid carcinoma cells also resulted in inhibition of the EGF-induced tyrosine phosphorylation of various cellular proteins including, the EGF receptor and phospholipase C-γ1 (Bae *et al*., 1997). These results suggested that H_2O_2 may act as a second messenger molecule.

Reversible inactivation of the PTPase family members by H_2O_2 may explain why the production of H_2O_2 is required in many cases for receptor-induced tyrosine phosphorylation. To investigate this possibility, the effect of H_2O_2 produced in response to EGF on the activity of protein tyrosine phosphatase 1B (PTP1B) was investiga-ted in A431 (Lee *et al*., 1998). H_2O_2 inactivated recom-binant PTP1B *in vitro* by oxidizing its catalytic site cysteine, most likely to sulfenic acid. The oxidized enzyme was reactivated more effectively by thioredoxin than by gluta-redoxin or glutathione at their physiological concentrations. Oxidation by H_2O_2 prevented modification of the catalytic cysteine of PTP1B by iodoacetic acid, suggesting that it should be possible to monitor the oxidation state of PTP1B in cells by measuring the incorporation of radio-activity into the enzyme after lysis of the cells in the presence of radiolabeled iodoacetic acid. The amount of such radioactivity associated with PTP1B immunoprecipitated from A431 cells that had been stimulated with EGF for 10 min was 27% less than that associated with PTP1B from unstimulated cells. The amount of iodoacetic acid-derived radioactivity associated with PTP1B reached a minimum 10 min after stimulation of cells with EGF and returned to baseline values by 40 min, suggesting that the oxidation of PTP1B is reversible in cells.

On the basis of the previous observation that growth factor-induced protein tyrosine phosphorylation requires H_2O_2 production and this current observation that growth factor-induced generation of H_2O_2 is sufficient to cause inactivation of PTP1B, it was proposed that the activation of a receptor PTK by interaction with a growth factor may not be sufficient to increase the steady state level of protein tyrosine phosphorylation in a cell; rather, concurrent inhibition of PTPs by H_2O_2 may also be required for this effect. The extent of tyrosine phosphorylation of receptor PTKs and their substrates would then return to basal values after degradation of H_2O_2 and the subsequent reactivation of PTPs by electron donors. *In vitro* data suggested that Trx might be a physiological electron donor for PTP1B. This was the first direct demonstration linking the oxidation of protein Cys residues to signaling, and confirmed the hypothesis that reversible oxidation by H_2O_2 is a means of signal propagation. This hypothesis is also consistent with the suggestion that the ligand-independent basal activity of receptor PTKs might be sufficient to increase the extent of protein tyrosine phos-phorylation in cells treated with thiol-alkylating agents, such as iodoacetic acid and iodoacetamide, or oxidants, such as ultraviolet light, that cause the inactivation of PTPs (Knebel *et al*., 1996). Likewise, reversible oxidation of the essential Cys residues of Caspase family members, which are implicated in apoptosis, may account for the observed role of H_2O_2 in apoptosis. Ras is also likely a target of H_2O_2: Recently, Cys118 of Ras was shown to be modified by NO, triggering downstream signaling events (Lander *et al*., 1996).

Both prokaryotic and higher eukaryotic cells are able to alter their genetic program in response to changes in the intracellular levels of ROS. In bacteria and yeast, this response leads to the new synthesis of proteins that protects cells from the consequences of oxidative damage. The resposne to oxidative stress has been extensively studied in *E. coli* (Rosner and Storz, 1997). In *E. coli* two transcriptional factor systems, SoxR and OxyR, have been identified that activate expression of genes whose products are involved in either the protection from oxi-dative stress or the repair of ROS-mediated damage. Intriguingly, the two transcriptional factors respond to different ROS species. SoxR/R with iron-sulfur center is activated by $O_2^{\cdot-}$ (Hidalgo *et al*., 1997), whereas OxyR is selectively induced by H_2O_2 (Zheng *et al*., 1998). *In vitro* and *in vivo* studies of oxidized OxyR revealed that the transcriptional factor is activated through the formation of a disulfide bond and is deactivated by reduction with glutaredoxin (Zheng *et al*.,

1998).

NF-κB was the first eukaryotic transcription factor shown to respond directly to oxidative stress (Schreck *et al*., 1991; Baeuerle and Baltimore, 1996; Kretz-Remy *et al*., 1996). The transactivator plays a crucial role in the regulation of numerous genes involved in immune and inflammatory processes. Potent stimuli activating NF- kB in intact cells are the TNF-α and IL-1, phorbol ester, LPS, and UV radiation. Interestingly, many, if not all NF- κB-inducing agents lead to an increase in the intracellular concentration of ROS. Furthermore, exposure of several types of cells to H_2O_2 rapidly induced NF- kB activation, sugggesting that H_2O_2 might be the mediator of prooxidant-induced NF- kB activation. As frequently speculated in literature, the essential Cys residues of transcriptional factors (c-fos, c-jun, NF- κB, hypoxia-inducible transcription factor-1) are likely targets of H_2O_2 modification and this modification together with phosphorylation events may be responsible for their complex signaling mechanisms. In addition to the predicted target proteins mentioned above, there are likely many more target proteins to be discovered.

Elimination of H_2O_2

Conventional antioxidant enzymes like superoxide dismutase, catalase, glutathion peroxidase and small antioxidant molecules like a-tocopherol, lipoic acid, ascorbic acid, and uric acid are believed to exist in order to remove ROS or repair damage caused by ROS. The roles of these antioxidant molecules in cell function have been studied extensively with the hope of gaining an upper hand against various human diseases and to fight the process of aging. However, given the recent indications on the messenger role of H_2O_2, many investigators in the area of antioxidant defense have turned their attention to cell signaling and have suggested certain roles for various antioxidants.

Our interest in H_2O_2 was triggered by our discovery of a thioredoxin-dependent peroxidase (TPx) from yeast that reduces H_2O_2 with thioredoxin (Trx) as an immediate electron donor (Chae *et al*., 1993; Chae *et al*., 1994; Chae *et al*., 1994; Kim *et al*., 1998). A database search revealed more than 40 proteins from a wide variety of species that show sequence similarity to yeast TPx (Chae *et al*., 1994). These homologous proteins were named the peroxiredoxin (Prx) family (Chae *et al*., 1994). They were not termed the TPx family because not all members use Trx as the hydrogen donor. Initially, we regarded the yeast enzyme to be another antioxidant enzyme that simply protected cellular components from oxidative damage and we called it thiol-specific antioxidant (TSA) (Chae *et al*., 1993; Chae *et al*., 1994). Subsequently we found that like many proteins involved in signal transduction, Prx exists as mutiple (at least six) isoforms in mammalian cells. Furthermore, several of the Prx isoforms were discovered in connection with cellular functions such as proliferation, differentiation, natural killer cell activity, osteoregulation, and keratinocyte function. These seemingly unrelated functions, which were reported without reference to any peroxidase activity, led us to hypothesize that Prx proteins participate in the signaling cascade of various receptors by regulating the intra-cellular concentration of H_2O_2.
A is exemplified by the examples of cAMP, cGMP, and Ins 1,4,5-P3, the controlled removal of second messenger molecules is an important feature of receptor signaling. To date, catalase and GPx have been viewed as the major enzymes responsible for removal of cytotoxic H_2O_2. However, catalase is largely or entirely localized in peroxisomes and GPx is present mainly in mitochondria and nuclei. Additionally, small molecular antioxidants like a-tocopherol, lipoic acid, ascorbic acid, and uric acid show low reducing activity toward H_2O_2. In contrast, five of the six Prx isoforms are abundant in the cytosol and are found in almost every tissue.

We investigated the cellular function of mammalian Prx members by transiently expressing them in various cultured cells. Overexpression of a wild-type Prx in NIH 3T3 cells and A431 cells completely overcame the H_2O_2 increase in response to growth factor and inhibits the activation of c-jun N-terminal kinase (JNK) induced by H_2O_2 or growth factors, whereas expression of the inactive mutant lacking the active Cys had no effect (Jin *et al*., 1997; Kang *et al*., 1998; Kang *et al*., 1998). Furthermore, overexpression of a peroxiredoxin in HeLa cells inhibited both the NF-κB activation and apoptosis elicited by H_2O_2 or TNF-α (Jin *et al*., 1997; Zhang *et al*., 1997; Kang *et al*., 1998; Kang *et al*., 1998). These results suggest that Prx proteins act as a regulator of intracellular H_2O_2 concentration and signal transduction. These conclusions are consistent with the fact that Prx proteins were discovered in connection with a variety of seemingly unrelated cellular processes such as proliferation, natural killer cell activity, the response to oxidative stress, and osteoregulation. An immediate question to be addressed is how the peroxidase activity of Prx enzymes is modulated in response to receptor occupancy.

Concluding remarks

Over the next several years,H_2O_2 is expected to join the ranks of cAMP, Ca^{2+}, Ins 1,4,5-P3, and NO in its role as a second messenger as a result of the contribution of many laboratories throughout the world. A plethora of new scientific principles have been established through studies on the mechanisms for the production and elimination of the messengers and during identification of downstream targets. History shows that advances in the basic understanding of cell signaling have invariably led to the means of developing new theraputic reagents. Studies on H_2O_2 will be no exception to this legacy of discovery and application.

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