ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 9 & 10, 2006

Forum Editorial

Reactive Oxygen Intermediates and Signaling Through Kinase Pathways

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CELLS, WHETHER PHOKARYOTE, ANIMAL, OR PLANT, have evolved under the constant stress of oxygen radicals. Oxygen radicals can be generated in cells via both respiration and photosynthesis (3, 8). In addition, UV light, triggering of certain cell-surface receptors, and exposure to a variety of chemicals will result in the generation of intracellular oxygen radicals (11, 16, 36, 39). Extrinsic exposure to reactive oxygen intermediates also can occur (15). Oxygen intermediates can be harmful to the cell, as they can damage DNA, proteins, and lipids (34).

All cells sense and respond to their environment. Often this function is performed using ligand receptors, which trigger the activation of cellular kinases that in turn result in changes in enzymatic activity or in the induction of transcription factors. Cells must also sense the presence of free radicals and respond appropriately to them. Plants, animals, and bacteria respond to reactive oxygen intermediates in their environment. One mechanism by which cells will respond to reactive oxygen intermediates is to modify the activity of certain cellular signaling pathways.

Clearly, some selective pressure exists to promote cell survival in response to reactive oxygen intermediates. In response to oxygen radicals, cells are known to upregulate antioxidant pathways in bacterial (35), plant (8), or animal cells (20). In multiple-cell organisms, inducing cellular death of a single damaged cell could benefit the survival of the organism. Reactive oxygen intermediates are known to induce cellular signaling pathways in multiple-cell organisms that result in either the prevention of (12, 19, 28) or induction of apoptosis (4, 14, 22, 25, 26, 44). Similar mechanisms of activation/inactivation of kinases can occur between types of organisms and also within cellular signaling pathways. Histidine kinases are activated in response to oxidative stress in plants and bacteria (2, 40). Mitogen activated protein kinase (MAPK) signaling pathways are activated by oxidative stress and can be found in plants and animal cells (9, 43). Inactivation of phosphatases, as a mechanism to activate these kinase pathways, may also be conserved across plants and animals (13, 28, 31). This Forum issue of *Antioxidants and Redox Signaling* highlights the influence of reactive oxygen intermediates on protein kinases in both plants and animals and highlights potential mechanisms by which this may occur.

In this Forum, Andrea Pitzcheke *et al.* (33) review the effects of reactive oxygen intermediates on cellular signaling in plants, where they mediate responses such as stromal closure, root hair development, and pathogen defense. The majority of the intracellular reactive oxygen intermediates in plants are produced in the peroxisomes and chloroplasts. Pitzcheke *et al.* (33) review the role of reactive oxygen intermediates in pathogen defense, where they appear to activate signaling pathways that lead to apoptosis or cell death much as they do in animal cells. The apoptotic response is thought to prevent the spread of the pathogen and when the respiratory burst oxidase homolog (*rboh*) gene is knocked out in *Arabidopsis*; decreases in reactive oxygen intermediates and apoptosis are noted in response to bacterial challenge (38).

In some cases, the intracellular signaling pathways activated by reactive oxygen species in plants can be very similar to those found in animals. Pitzcheke *et al.* (33) discuss how reactive oxygen intermediates have a role in activating the MAPK kinase pathway in *Arabidopsis*. This activation occurs via an NPK1-related protein kinase (ANP1)/MAPK pathway and appears to protect *Arabidopsis* from cellular stress. It is thought that the activation of this pathway protects cells from cellular stresses such as oxidative, cold, and salt stress, much as the extracellular regulated kinase (ERK), big MAP kinase (BMK1), and Jun-N-terminal kinase (JNK) pathways can protect animal cells from different cellular stresses (reviewed by McCubrey *et al.* in this issue, 29). Regulation of the MAPK pathway in plants may occur in part due to reactive oxygen intermediate–induced inactivation of phosphatases, as hydrogen peroxide has been shown to has been demonstrated to inhibit the plant phosphatase PTP1 (13).

The review in this Forum, written by Nick Leslie (27), examines the ability of reactive oxygen intermediates to activate a very important antiapoptotic pathway in animal cells,

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the phosphatidylinositol-3-kinase (PI3K) pathway. Reactive oxygen species–induced regulation of the PI3K signaling pathway appears to occur at multiple points within this pathway. Hydrogen peroxide–induced activation of the PI3K pathways appears to occur in part because of the inactivation of the phosphatase and tensin homolog phosphatase, which opposes PI3K activation (28). Additionally, Leslie (27) discusses the ability of protein phosphatase 2A (PP2A) and Akt, a downstream target of the PI3K pathway, to undergo oxidation directly. The oxidation of Akt was not shown to influence Akt activity *in vitro* but is proposed to inhibit the ability of Akt to interact with PP2A, favoring Akt activation (41). Leslie also discusses the ability of nitric oxide to influence the association between the p85 and p110 subunits of PI3K (6). The disassociation of p85 and p110 is predicted to prevent PI3K activation and lead to an increased sensitivity to apoptosis.

McCubrey *et al.* (29) reviews the literature that demonstrates that multiple species of reactive oxygen intermediates can modulate phosphorylation within the MAPK pathways. Specifically, they examine the effects of superoxide anion, peroxides, and nitric oxide on the ERK, JNK, p38, and BMK pathways. In particular, these authors discuss both the anti-apoptotic and apoptotic effects of signaling by these pathways and the effects of both endogenously and exogenously produced oxygen radicals on these pathways. Pantano *et al.* (32) discuss the ability of reactive oxygen intermediates to activate another anti-apoptotic pathway, the nuclear factor-κB (NF-κB) signaling pathway. Furthermore, these authors address the ability of reactive oxygen intermediates directly to activate IKK, a very important protein leading to the induction of NF-κB activity. In addition, these authors review the literature that demonstrates that reactive oxygen intermediates can induce activation of the NF-κB pathway via their aforementioned ability to modulate the PI3K and JNK signaling pathways. The review by Franklin *et al.* (10) reports on the ability of hydrogen peroxide to activate the CaM-kinases (18). These authors report that this activation is likely to occur via phosphatase inhibition, as phosphatase inhibitors can also induce CaM-K activation (18). Furthermore, these authors demonstrate that, similar to the earlier mentioned pathways, the reactive oxygen intermediate–induced CaM-K pathway may contribute to the activation of the antiapoptotic PI3K, NF-κB, and ERK signaling pathways (17).

The article by Cross and Templeton (5) is especially interesting in that these authors demonstrate a novel mechanisms for the regulation of these signaling pathway. Cross and Templeton (5) review the mechanisms by which oxidation of the free cysteine residues can occur and the effects of this direct oxidation on a number of different kinases. In addition, they review recent literature, which demonstrates that free cysteine groups can undergo glutathionylation in response to oxidative stress. Furthermore, they discuss both the positive and negative effects that glutathionylation has on specific enzymes and the role that this may play in cell survival.

The reviews in this FORUM indicate that cells of all kingdoms (plant, animal, or bacterial) respond to the presence of reactive oxygen intermediates by activating or inhibiting a variety of cellular signaling kinases. Although some of these pathways may be very similar between the kingdoms, reactive oxygen intermediates can influence the signaling of a wide variety of kinase pathways, such as the MAPK, NF-κB, CaM-K, and PI3K pathways. These kinase pathways can have both antiapoptotic and apoptotic roles. One mechanism by which modulation of cellular signaling pathways by reactive oxygen intermediates can occur is via the modification of sulfhydryl groups. This can occur directly by the oxidation of these groups, causing disulfide bond formation or sulfinic acids. Oxidation of protein and lipid phosphatases by reactive oxygen intermediates has been proposed as one mechanism by which modulation of the kinase signaling pathways can occur. However, this is clearly not the only mechanism by which modulation of these pathways can occur, as glutathionylation and oxidation of the kinases themselves can influence their activity. In the future, it will be important to determine whether glutathionylation also occurs in plants cells, whether modulation of these pathways can influence the outcome of exposure to certain reactive oxygen intermediate–inducing treatments, and the relative contribution of phosphatase inactivation, glutathionylation, and in the activation of the different kinases.

ACKNOWLEDGMENTS

J.A.M. and R.A.F. were supported in part by a grant from the National Institutes of Health (R01 CA98195). J.A.M. was supported in part by a grant from the National Institutes of Health (R01 CA51025). R.A.F. was supported in part by a Grant-in-Aid (0355834U) from the American Heart Association.

ABBREVIATIONS

BMK1, big MAP kinase; ERK, extracellular regulated kinase; JNK, Jun-N-terminal kinase; MAPK, mitogen-activated protein kinase; ANP1, NPK1-related protein kinase; NF-κB, nuclear factor-kappa B; PTEN, phosphatase and tensin homolog; PI3K, phosphatidylinositol-3-kinase; PP2A, protein phosphatase 2A.

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First submission to ARS Central, April 27, 2006; date of acceptance, May 2, 2006.

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