

## Minireview

# Redox-dependent signal transduction

Toren Finkel\*

Laboratory of Molecular Biology, National Heart Lung and Blood Institute, National Institutes of Health, Bldg 10/6N-240, 10 Center Drive, Bethesda, MD 20892-1622, USA

Received 5 May 2000

Edited by Gunnar von Heijne

**Abstract** Reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide appear to be transiently produced in response to growth factor and cytokine stimulation. A variety of evidence suggests that this ligand-stimulated change in the cellular redox state participates in downstream signal transduction. This review will focus on the effects of ROS on signal transduction pathways, the molecules that regulate intracellular ROS production and the potential protein targets of oxidants. © 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

*Key words:* Signal transduction; Redox; Peroxide; Rac1; NADPH oxidase

### 1. Oxidants as signaling molecules

The cascade of events triggered by a growth factor or cytokine binding to its cognate receptor has been the subject of intense scrutiny for the last several decades. In the last several years it has become increasingly evident that ligand-stimulated reactive oxygen species (ROS) generation plays a role in the complex world of signal transduction [1–3]. Analysis of cells in culture has demonstrated that a variety of ligands including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), angiotensin II, as well as a host of cytokines, all trigger the rapid production of intracellular ROS [4–8].

Following the initial descriptions that certain ligands activate ROS production, the question remained as to whether this phenomenon represented a toxic or non-specific response, or whether the generation of ROS represented an important element in signaling pathways. Relatively little is known regarding the oxidase(s) that may be regulated in a ligand-dependent fashion. As such the initial studies assessing the role of oxidants in signal transduction have relied on the overexpression of antioxidant scavenging proteins or treatment with chemical scavengers. These initial studies demonstrated that inhibiting the rise in ROS levels following ligand addition inhibited a variety of downstream pathways. In particular, increasing the level of the peroxide scavenging protein catalase was shown to inhibit the ability of PDGF to stimulate tyrosine phosphorylation of a number of proteins [7]. A similar approach with EGF yielded similar results [6]. This phenomenon of redox-dependent signaling is not however limited to factors that act through tyrosine kinase receptors since angio-

tensin II signal transduction is also affected by redox modulation [9].

### 2. Regulation of non-phagocytic oxidase(s)

Although these results suggest a role for ROS in a diverse set of signaling pathways it is important to note that most if not all of these studies have relied on broad acting antioxidants. As such, it became critical to define the precise way in which ligands could activate ROS production. In phagocytic cells, a variety of stimuli cause the massive release of superoxide anion through the assembly of NADPH oxidase. This multimeric protein complex is essential for neutrophil function and is composed of both membrane-bound proteins and cytosolic factors [10]. One essential component for assembly of the neutrophil NADPH oxidase is the small GTPase rac2. Although rac2 is expressed primarily in phagocytic cells, the homologous rac1 protein is more ubiquitously expressed. Consistent with the notion that the rac family of proteins possess the ability to regulate ligand-stimulated ROS production in a variety of cell types is the relatively recent observation that activating mutants of rac1 stimulate ROS production in fibroblasts and other non-phagocytic cell types [8,11–14]. Similarly, expression of the dominant negative form of rac1 in non-phagocytic cells appears to inhibit ROS production following stimulation by a variety of different ligands [8,11]. Interestingly, the ability of rac proteins to contribute to ras-mediated transformation appears in part related to their ability to produce superoxide anions [12,13]. In particular, site-directed mutants of rac that specifically inhibit superoxide production fail to stimulate DNA synthesis [13].

The ability of rac to regulate superoxide production in both phagocytic and non-phagocytic cells suggests that non-phagocytic cells may have a molecular oxidase complex similar to the NADPH oxidase of neutrophils. This is supported by biochemical studies suggesting NADPH/NADH oxidase activity can be detected in a variety of cell lysates [15]. Although many components of the NADPH oxidase are ubiquitously expressed, the major cytochrome, gp91, appears unique to phagocytes. Nonetheless, two recent reports have revealed a homologue of gp91 that is expressed in non-phagocytic cells [16,17]. Evidence suggests that this protein might participate in growth regulation [16]. This suggests that similar to neutrophils, non-phagocytic cells appear to have a rac-regulated NADPH oxidase complex that produces ROS in response to a host of ligands. In many ways this suggests that analogous to another oxidant, nitric oxide, superoxide (and/or hydrogen peroxide) can be produced in immune effector cells in large quantities and in non-phagocytic cells in much smaller quan-

\*Fax: (1)-301-402 9311.  
E-mail: finkelt@nih.gov

tities. In immune cells, large scale production of NO by macrophages, or superoxide by neutrophils, provides a necessary host defense function, while when produced in smaller doses in non-phagocytic cells, these same reactive molecules function as signaling molecules. For both NO and superoxide, the complexes responsible for large scale immunological and smaller scale signaling functions represent homologous but distinct enzyme systems. In the case of NO, there are the inducible versus the constitutive nitric oxide synthase enzymes, while for the case of superoxide there appear to be the two similar, but structurally distinct, NADPH oxidase systems.

### 3. Mechanism of oxidant signaling

Although the above sets of experiments implicate ROS as mediators of growth factor signal transduction, relatively little is known regarding the specific mechanism through which oxidants act. In particular, given the exquisite nature of signaling pathways it would seem likely that once generated, ROS must in some way intersect with the known and well established pathways governing cell growth. This would suggest that oxidants may have direct protein targets wherein exposure of these proteins to an altered redox state alters the function of the target protein. Some recent evidence supports this notion and suggests that both certain protein–protein interactions and enzymatic function might be regulated by cellular ROS levels.

One piece of evidence linking the cellular redox state to specific signaling pathways came from the analysis of a yeast two-hybrid screen using apoptosis signal-regulated kinase 1 (ASK1) as bait. ASK1 is a member of the family of mitogen-activated protein kinase kinases that is involved in the activation of stress-activated protein kinases (SAPK or c-Jun NH<sub>2</sub>-terminal kinases) and p38 kinase. Interestingly, thioredoxin, an antioxidant protein, was found to form a complex with ASK1. When thioredoxin is complexed to ASK1, the activity of ASK1 is inhibited [18]. The rise in ROS levels that occurs following tumor necrosis factor stimulation resulted in the dissociation of ASK1 from thioredoxin and the subsequent activation of ASK1 activity [18,19]. As such, thioredoxin can be thought of as both an antioxidant protein and a redox sensor. It is unlikely that this dual function is unique for thioredoxin and as such the concept of antioxidant proteins functioning only as free floating ROS scavengers may need to be revised. Indeed it is possible that other antioxidant proteins such as the periredoxin family [20] will also be shown to interact with specific intracellular signaling molecules and regulate their activity in a redox-dependent fashion. Interestingly, also consistent with this notion, previous studies have determined that superoxide dismutase (Sod1) can interact and regulate calcineurin activity [21].

Another way that changes in ROS can alter signaling pathways is by specifically altering the oxidation of reactive cysteine residues in proteins. Under physiological conditions, most cysteines in protein are in the protonated (SH) form. In certain proteins, certain cysteines have their sulfhydryl group oxidized at physiological pH. The exact basis for formation of what is termed a reactive cysteine is presently unclear although it appears to require the contribution of positive charges from neighboring amino acids. Perhaps the best characterized reactive cysteine is found in the bacterial tran-

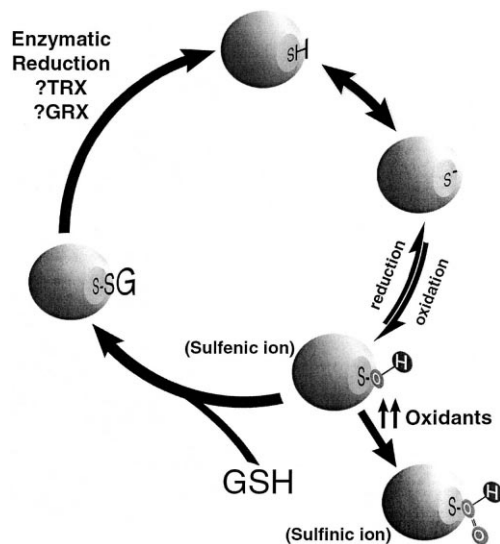


Fig. 1. Proposed pathway for signal transduction by reactive cysteine modification. Most cysteine residues in proteins are fully reduced at physiological pH, however, certain reactive cysteine residues, most likely due to the contribution of positive charges from neighboring amino acids, can be readily oxidized at pH 7 or below. Further oxidation leads to the formation of a sulfenic ion and in the case of tyrosine phosphatases to a corresponding reduction in enzymatic activity. Continued high levels of oxidant stress would lead to further and most likely irreversible oxidation to a sulfonic ion. In contrast, the sulfenic ion formed by mild oxidative stress can be reduced back to S<sup>-</sup>. Given the presence of millimolar glutathione (GSH) inside cells, one likely pathway for reduction would involve the formation of a protein mix disulfide. These protein mix disulfides could then in turn be substrates for enzymes such as thioredoxin (Trx) or glutaredoxin (Grx) to return the reactive cysteine to its basal state. Such a pathway would allow for the transient, specific and reversible modification of enzymatic activity in proteins with a reactive cysteine. In many ways, this is analogous to the way phosphorylation of specific amino acids regulates enzymatic activity.

scription factor OxyR whose activity is sensitive to the redox state [22]. Nonetheless, a variety of proteins in mammalian cells have been demonstrated to contain reactive cysteines. From a signaling point of view perhaps the most relevant is the family of proteins that act as protein tyrosine phosphatases. All members of this family have a reactive cysteine in their active site and it has been appreciated for some time that oxidant stress can reversibly inactivate cellular tyrosine phosphatase activity. The mechanism for transient inactivation presumably involves oxidation of the reactive cysteine to form a sulfenic ion. In this form, the enzymatic activity of the phosphatase (or potentially other enzymes) is significantly reduced or abolished. The oxidized reactive cysteine now in the sulfenic form is most likely capable of interacting with cellular glutathione to form a protein mix disulfide. This mix disulfide can be reduced probably through the actions of cellular enzymes such as glutaredoxin or thioredoxin to restore the original molecule. While continued high levels of oxidant stress could lead to irreversible oxidized states above sulfenic acid (i.e. creation of a sulfonic ion), the scheme diagrammed in Fig. 1 represents a specific, reversible mechanism for regulation of enzyme activity by oxidant stress. In this scenario, specificity comes from the local amino acid environment surrounding the cysteine residue that leads to its reactivity. In many ways this is analogous to amino acid motifs in

other proteins surrounding specific serine, threonine or tyrosine residues that serve as recognition for phosphorylation by cellular kinases. In both cases, enzymatic activity is regulated in a reversible fashion by targeting specific amino acids using either phosphate addition for the well characterized protein phosphorylation or cysteine oxidation/glutathione addition for redox signaling.

Evidence is slowly emerging that ligand-stimulated ROS production may be sufficient to regulate the specific activity of certain protein targets. Analysis of the protein tyrosine phosphatase 1B (PTP-1B) following EGF stimulation suggests a temporary inactivation of phosphatase activity that corresponds temporally with the burst of ROS production [23]. Interestingly, analysis of PTP-1B reactive cysteine moiety following EGF stimulation demonstrates a transient glutathiolation of PTP-1B at the peak of ROS production [24]. This suggests that the covalent addition of glutathione to reactive cysteines may be one mechanism for achieving reversible redox-dependent signaling as well as a potential means for identifying relevant redox-dependent signaling molecules.

In summary, emerging evidence suggests the participation of reactive oxygen species as a component of signal transduction for a wide host of growth factors and cytokines. Further studies are needed to define the exact chemical nature of the relevant ROS involved in signal transduction, the enzymatic intracellular source of ligand-stimulated ROS and the specific protein targets of oxidants. Given that oxidant stress appears to contribute to a variety of human diseases, it seems likely that the information learned from probing oxidant signaling will provide insight into a wide variety of physiological and pathophysiological conditions.

## References

- [1] Finkel, T. (1998) *Curr. Opin. Cell Biol.* 10, 248–253.
- [2] Rhee, S.G. (1999) *Exp. Mol. Med.* 31, 53–59.
- [3] Lander, H.M. (1997) *FASEB J.* 11, 118–124.
- [4] Speir, E., Modali, R., Huang, E.S., Leon, M.B., Shawl, F., Finkel, T. and Epstein, S.E. (1994) *Science* 265, 391–394.
- [5] Griendling, K.K., Minieri, C.A., Ollerenshaw, J.D. and Alexander, R.W. (1994) *Circ. Res.* 74, 1141–1148.
- [6] Bae, Y.S., Kang, S.W., Seo, M.S., Baines, I.C., Tekle, E., Chock, P.B. and Rhee, S.G. (1997) *J. Biol. Chem.* 272, 217–221.
- [7] Sundaresan, M., Yu, Z.X., Ferrans, V.J., Irani, K. and Finkel, T. (1995) *Science* 270, 296–299.
- [8] Sundaresan, M., Yu, Z.X., Ferrans, V.J., Sulciner, D.J., Gutkind, J.S., Irani, K., Goldschmidt-Clermont, P.J. and Finkel, T. (1996) *Biochem. J.* 318, 379–382.
- [9] Ushio-Fukai, M., Zafari, A.M., Fukui, T., Ishizaka, N. and Griendling, K.K. (1996) *J. Biol. Chem.* 271, 23317–23321.
- [10] DeLeo, F.R. and Quinn, M.T. (1996) *J. Leukocyte Biol.* 60, 677–691.
- [11] Sulciner, D.J., Irani, K., Yu, Z.X., Ferrans, V.J., Goldschmidt-Clermont, P. and Finkel, T. (1996) *Mol. Cell. Biol.* 16, 7115–7121.
- [12] Irani, K., Xia, Y., Zweier, J.L., Sollott, S.J., Der, C.J., Fearon, E.R., Sundaresan, M., Finkel, T. and Goldschmidt-Clermont, P.J. (1997) *Science* 275, 1649–1652.
- [13] Joneson, T. and Bar-Sagi, D. (1998) *J. Biol. Chem.* 273, 17991–17994.
- [14] Kheradmand, F., Werner, E., Tremble, P., Symons, M. and Werb, Z. (1998) *Science* 280, 898–902.
- [15] Griendling, K.K. and Ushio-Fukai, M. (1997) *Trends Cardiovasc. Med.* 7, 301–307.
- [16] Suh, Y.A., Arnold, R.S., Lassegue, B., Shi, J., Xu, X., Sorescu, D., Chung, A.B., Griendling, K.K. and Lambeth, J.D. (1999) *Nature* 401, 79–82.
- [17] Banfi, B., Maturana, A., Jaconi, S., Arnaudeau, S., Laforge, T., Sinha, B., Ligeti, E., Demaurex, N. and Krause, K.H. (2000) *Science* 287, 138–142.
- [18] Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K. and Ichijo, H. (1998) *EMBO J.* 17, 2596–2606.
- [19] Gotoh, Y. and Cooper, J.A. (1998) *J. Biol. Chem.* 273, 17477–17482.
- [20] Rhee, S.G., Kang, S.W., Netto, L.E., Seo, M.S. and Stadtman, E.R. (1999) *Biofactors* 10, 207–209.
- [21] Wang, X.T., Culotta, V.C. and Klee, C.B. (1996) *Nature* 383, 434–437.
- [22] Zheng, M., Aslund, F. and Storz, G. (1998) *Science* 279, 1718–1721.
- [23] Lee, S.R., Kwon, K.S., Kim, S.R. and Rhee, S.G. (1998) *J. Biol. Chem.* 273, 15366–15372.
- [24] Barrett, W.C., DeGnore, J.P., Keng, Y.F., Zhang, Z.Y., Yim, M.B. and Chock, P.B. (1999) *J. Biol. Chem.* 274, 34543–34546.