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Previews

Oxidants Painting the Cysteine Chapel: Redox Regulation of PTPs

Growth factors and cytokines appear to stimulate the intracellular production of reactive oxygen species (ROS). Evidence suggests that this alteration in the cellular redox state is essential for downstream signaling, but the precise mechanism has remained elusive. A new study now demonstrates that ligand-stimulated intracellular hydrogen peroxide can specifically and reversibly regulate the activity of protein tyrosine phosphatases.

Although generally viewed solely as a necessary evil of aerobic metabolism, the generation of intracellular ROS has gone through considerable reevaluation in the last decade. It has long been appreciated that phagocytic cells, such as neutrophils and macrophages, purposely produce ROS for their specialized function as immune mediators. More surprising, however, were the observations made in a variety of nonphagocytic cells demonstrating that ligands such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) can stimulate the rapid and transient production of oxidants (Sundaresan et al., 1995; Bae et al., 1997). Interestingly, these initial studies demonstrated that ligand-stimulated ROS generation was in fact required for growth factor-induced tyrosine phosphorylation. These observations complemented a large body of previous literature demonstrating that, in some settings, the simple addition of exogenous hydrogen peroxide was sufficient to augment intracellular tyrosine phosphorylation levels and to induce cellular proliferation.

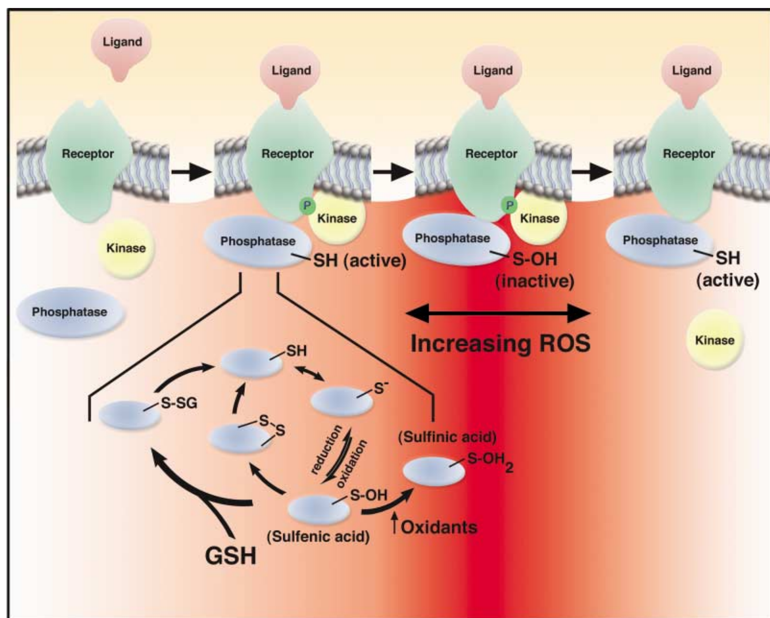
Missing from these earlier studies, however, was a convincing mechanistic explanation for how oxidants could effect downstream signaling. Although the initial hypothesis centered on transient inactivation of tyrosine phosphatases (Sundaresan et al., 1995), proof was lacking. Now, a new study by Meng et al., appearing in the February issue of *Molecular Cell*, goes a long way toward answering these mechanistic questions and, in doing so, provides a new and useful assay to uncover additional targets of oxidant-mediated signaling (Meng et al., 2002).

Protein tyrosine phosphatases (PTPs) represent a large family of both soluble and membrane-bound enzymes (Tonks and Neel, 2001). Although significant progress has been made in demonstrating that these enzymes exhibit considerable substrate specificity, less information is available on the regulation of their enzymatic activity. Common to the active site of all tyrosine or dual-specific phosphatases is an invariant active site cysteine residue. Unlike most cysteine residues within proteins, which remain protonated at physiological pH ($pKa > 8.0$), the catalytic site cysteine residue of tyrosine phosphatases is extremely reactive and rapidly forms a thiolate anion at physiological pH ($pKa < 6$). The reactivity of this cysteine is in fact essential for the catalytic mechanism of tyrosine phosphatases, which requires a phospho-Cys intermediate. Nonetheless, the very reactivity required for enzymatic activity in turn renders the catalytic cysteine vulnerable to oxidation. Studies with purified enzymes have suggested that hydrogen peroxide treatment readily inactivates a number of PTP family members while having no effect on serine/threonine phosphatases (Denu and Tanner, 1998). In these studies, mild hydrogen peroxide treatment resulted in the formation of a sulfenic acid (Cys-SOH) intermediate. This relatively unstable intermediate could, however, be reversed with the addition of a thiol-reducing agent such as glutathione.

The new study by Meng and colleagues has cleverly taken advantage of the chemistry of reactive cysteines. In particular, the authors have developed a novel assay that allows for the discrimination of oxidized and hence inactive PTPs from phosphatases that are reduced and therefore enzymatically active. The assay employs iodoacetic acid (IAA), which is capable of irreversibly alkylating reactive cysteine residues but cannot alkylate cysteine residues that have been previously oxidized to their sulphenic acid derivative. Combining this approach with the more standard in-gel PTP activity assay, the authors were able to conclusively demonstrate that in vivo a number of PTPs are inactivated by exogenous hydrogen peroxide treatment. In addition, under more physiological conditions, PDGF stimulation of Rat-1 cells resulted in a burst of ROS and the rapid inactivation of a 70 kDa protein subsequently identified as the protein tyrosine phosphatase SHP-2. Interestingly, PTP recruitment to the PDGF receptor (PDGFR) appears to be required for ROS-induced inactivation. The authors suggest that this is the result of a conformational change of SHP-2 upon receptor binding that renders the molecule more susceptible to ROS attack, although it is also possible that membrane recruitment places the PTP closer to the source of ligand-activated oxidant generation.

Previous studies have also hinted that PTPs may be regulated by the intracellular redox state. In particular, Rhee and colleagues demonstrated that EGF stimulation of A431 cells induces a burst of ROS whose time course inversely correlates with the activity of PTP1B (Lee et al., 1998). Subsequent studies demonstrated that the cysteine-sulfenic intermediate formed by oxidation of PTP1B could in the presence of glutathione form a mixed disulfide (Barrett et al., 1999). The formation of mixed disulfides, alternatively known as glutathionylated proteins, has recently led to an alternative approach for the identification of proteins directly modified by physiological oxidative stress (Sullivan et al., 2000).

Besides providing a novel assay, this new study also addresses another long-standing dilemma in signal transduction. Tyrosine kinase receptor activation and subsequent autophosphorylation is well known to create docking sites for a number of signaling molecules. In the case of the PDGFR, the recruited molecules include lipid and tyrosine kinases as well as PTPs such as SHP-2.



A Model for Redox Regulation of Protein Tyrosine Phosphatase Activity

Recruitment of tyrosine phosphatases such as SHP-2 to membrane receptors is stimulated by ligand engagement. Receptor-bound, fully active PTPs could in turn destroy binding sites for other signaling molecules that have been recruited simultaneously. The observed rise in intracellular ROS can, however, transiently inactivate PTP activity, presumably through the formation of a sulfenic intermediate. The presence of either glutathione (GSH) or another intramolecular reactive cysteine allows for reversibility by providing a pathway to reduce the oxidized cysteine.

Enzymatically active SHP-2 can, however, rapidly and efficiently remove phosphotyrosine residues, and in the process remove the newly created binding sites for signaling molecules such as PI3-kinase and Ras-GAP. How then does signal transduction ever happen? One potential solution would be to delay PTP activation, allowing other signaling molecules to act first. Meng and colleagues suggest that this form of molecular manners may be the basis for why so many ligands induce ROS generation. As depicted in the figure, the ROS-mediated inactivation of tyrosine phosphatases may provide a molecular "stun gun," allowing for simultaneous recruitment of a host of signaling molecules but temporal staggering of their enzymatic activity.

Finally, the role of reactive cysteine residues and sulfenic acid chemistry may extend well beyond the biology of PTPs. Indeed, similar mechanisms appear to regulate the activity of the oxidative stress-responsive bacterial transcription factor OxyR, the yeast peroxide sensor Yap1, and the DNA binding of the mammalian Fos and Jun transcription factors (Claiborne et al., 1999). Once formed, sulfenic acid intermediates can potentially be rescued by glutathione (see Figure) or, if another reactive cysteine is available, through the formation of an intramolecular disulfide bond. Like protein phosphorylation, this presumably ancient signaling system provides a specific and reversible mechanism to regulate enzymatic activity through the covalent modification of a target amino acid. For phosphorylation, specificity is achieved by surrounding the target serine, threonine, or tyrosine residue with additional amino acids to constitute a kinase recognition motif. For oxidant signaling, the target cysteine is again dependent on the surrounding amino acids, which provide the necessary local environment to lower the pKa and thereby provide reactivity. The development of novel assay systems, such as that described by Meng and colleagues, will go a long way in helping to define the precise molecular targets of intracellular oxidants. Future studies and additional approaches should help define the complete set of target molecules, the other mechanism by which oxidants modify protein targets, and the ways in which those modifications are reversed.

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