Subcellular Localization of Nox-Containing Oxidases Provides Unique Insight Into Their Role in Vascular Oxidant Signaling

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Originally, reactive oxygen species (ROS) were thought to be key participants in cellular injury. However, it is rapidly becoming realized that many of the individual species, such as hydrogen peroxide, are important mediators in a diverse array of cellular signaling processes. These processes include physiological regulation associated with the cellular sensing of oxygen tension, forces derived from stretch and shear, the control of cellular growth, and death.1-4 One of the most active areas under investigation is the control of cellular growth, associated with the cellular sensing of oxygen tension, forces derived from stretch and shear, the control of cellular growth, and death.1-4

Subcellular localization of the Nox-1 and Nox-4 subunit-containing oxidases in vascular smooth muscle cells on caveolae and focal adhesions, respectively. These observations have important implications for the organization of oxidant signaling mechanisms thought to be involved in the control of fundamental physiological processes, including the role of Nox-1 expression in promoting cell growth3-5 and roles for Nox oxidase activation by p47phox subunit binding in the sensing of cellular stretch forces.6

Redox Control by Each Nox Is Likely to Be Localized
Local concentrations of the cytosolic Cu,Zn-form of SOD (SOD-1) and substances such as NO will determine which species are involved in signaling. The diffusion properties of NO as a dissolved gas allow sites of elevated superoxide production to become an active subcellular site of peroxynitrite (ONOO) formation and a source of activation of signaling processes that originate from the generation of ONOO and its derived species (eg, nitrogen dioxide). Thus, high local levels of SOD should shift signaling to peroxide-linked mechanisms because of prevention of the generation of other superoxide-derived species or the actions of superoxide itself on other signaling systems.

Peroxide that is derived from Nox-containing oxidases will be metabolized by enzymes including catalase, glutathione peroxidase, and other heme peroxidases. Localized aspects of the metabolism of peroxide by these enzymes are likely to have a major influence on the signaling systems that are regulated by ROS and redox. One aspect of the influence of peroxide metabolizing systems is that they will control the concentration gradient of peroxide in the subcellular region where it is produced. Another important factor is that many of the signaling mechanisms most sensitive to peroxide are regulated by processes that originate from its metabolism by
specific peroxide-consuming enzymes.\textsuperscript{1,2} For example, the metabolism of peroxide by the heme peroxidase reaction of cyclooxygenase activates the metabolism of arachidonic acid to prostaglandins by this enzyme. The metabolism of peroxide by catalase can activate the production of cGMP by soluble guanylate cyclase. As the rate of metabolism of peroxide by glutathione peroxidases and thioredoxin peroxiredoxins increases, a localized gradient of oxidized glutathione, thioredoxin, and NADP (by the glutathione and thioredoxin reductase reactions) is created. Changes in the redox status of each of these molecules are likely to have a major influence on the subcellular localization of signaling systems regulated by redox. When Nox-derived superoxide promotes the formation of reactive NO-derived species, these species will potentially have a localized influence on signaling both by promoting redox regulation (eg, glutathione oxidation) and by the direct modification of protein function through oxidation, nitrosation, or nitration reactions. Thus, Nox-derived ROS have many ways of regulating localized signaling systems through alterations in the concentration gradients of the species generated and cellular redox systems such as glutathione and NADPH.

**Evidence for Localization of Specific Signaling Roles for Oxidases and ROS**

Evidence already exists for the compartmentalization within vascular smooth muscle of signaling through different stimuli of ROS generation. For example, although exogenous peroxide and posthypoxic reoxygenation simultaneously activate both cGMP-associated relaxing and prostaglandin-mediated contracting mechanisms that appear to be endothelium-independent in human placental veins, acute exposure to lactate appears to selectively stimulate only the relaxing mechanism activated through the generation of peroxide.\textsuperscript{1,2} Stretch activates a Nox oxidase–derived peroxide-mediated enhancement of force generation in bovine coronary arteries through an src-dependent epidermal growth factor receptor activation of extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase. However, these arteries express relaxing mechanisms when exposed to posthypoxic reoxygenation, exogenous peroxide, and lactate that are more sensitive to peroxide than the ERK-mediated contractile mechanism.\textsuperscript{1,2,6} These examples suggest that localized sources of ROS generation within vascular smooth muscle cells appear to be tightly coupled to the selective activation of signaling systems that could be located in the region of the specific oxidases that are involved in controlling the response observed.

**Potential Importance of the Subcellular Localization of Nox-1 and Nox-4**

Hilenski and colleagues\textsuperscript{5} discuss some of the potential implications of Nox localization. For example, the presence of Nox-1 in caveolae could be involved in growth-promoting actions by angiotensin type-1 receptors. Activation of angiotensin type-1 receptors is associated with translocation to caveolae sites where Nox-1 activation potentially occurs through signaling linked to protein kinase C, Src-family kinases (src), receptor tyrosine kinases, and G proteins (rac; Figure). The localization of Nox-4 in focal adhesions and in the nucleus could be associated with integrin-linked signaling (eg, stretch) and aspects of gene expression (eg, growth, differentiation, inflammatory reactions, senescence, and apoptosis), respectively. Previous work from this group has provided evidence for an initial activation of Nox oxidases by protein kinase C (which phosphorylates the cytosolic p47phox subunit, promoting binding to Nox) and an amplification mechanism of sustained oxidase activation through a rac pathway that involves activation of src by ROS, a transactivation of the epidermal growth factor receptor, and phosphatidylinositol-3-kinase.\textsuperscript{7} Almost all aspects of the organization of localized systems controlling the activity of each form of Nox and the signaling processes linked to the ROS produced by these oxidases remain to be elucidated. Signaling linked to low levels of oxidase activation are likely to participate in functional responses linked to the sensing of stretch and O\textsubscript{2} tension.

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**Diagram:**

- A new area for scientific investigation: what cellular mechanisms are localized to control the activation of each Nox-containing oxidase and redox-regulated signaling system regulated by ROS products of each oxidase? PKC indicates protein kinase C; AT-1, angiotensin type-1 receptors; PI3K, phosphatidylinositol-3-kinase; NF-\(\kappa\)B, nuclear factor-\(\kappa\)B; and AP1, activator protein-1.
More prolonged and/or extreme levels of oxidase activation are likely to control gene expression, growth, senescence, and apoptosis. Thus, the observation that Nox oxidases have specific subcellular localization sites provides a unique new insight into their role in multiple signaling processes.

References
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