NOXious Phosphorylation
Smooth Muscle Reactive Oxygen Species Production Is Facilitated by Direct Activation of the NADPH Oxidase Nox1

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NADPH oxidases of the Nox family are important sources of reactive oxygen species (ROS). Nox-derived ROS serve multiple functions in the organism, but in the healthy vascular system they are primarily signaling molecules. Numerous cytokines, growth factors, and hormones have been shown to stimulate Nox-dependent ROS production as essential step in their signal transduction. Seven catalytically active Nox proteins are present in the human genome. In the vascular system, the constitutively active Nox4 provides a basal redox tone to tune the antioxidant defense, whereas Nox1, Nox2, and Nox5 contribute to the stimulus-dependent acute ROS production. There are still uncertainties on the cell-specific expression patterns of these proteins and particularly little is known about Nox5, which it is missing in the rodent genome. Despite this, it is generally accepted that endothelial cells predominantly express Nox2, whereas in smooth muscle cells of greater arteries, mainly Nox1 is expressed. In resistance vessels and also in response to vascular stress, Nox1 and Nox2 are expressed throughout the vessel.3

The activity control of Nox enzymes differs between the homologues. For Nox1 and Nox2, it is primarily a consequence of protein–protein interactions. The general concept is that upstream signaling cascades converge to the small GTPase Rac1 and to the phosphorylation of the cytosolic organizing protein p47phox. On phosphorylation, p47phox binds to the plasma membrane and also binds to the activating proteins: Noxa1 or Noxo1.6,7 The consequence of this is a translocation of the activating protein to the large transmembrane catalytically active Nox protein, which is thereby activated8 (Figure 1).

In this issue of Circulation Research, Streeter et al9 report a totally novel mechanism controlling the activity of Nox1, which is phosphorylation by protein kinase C beta (PKCβ). The authors demonstrate that this event is also relevant for oxidative stress in vivo. In atherosclerosis, during neointima formation and in response to inflammatory cytokines, Nox1 becomes phosphorylated and active. Subsequent mass spec analyses and mutational studies led to the identification of the critical importance of threonine 429 in this process. The authors confirm previous work that interaction of Nox1 with cytosolic proteins is a prerequisite for Nox1 activation,2,6,7 but without Nox1 T429 phosphorylation this interaction cannot occur and stimuli, such as tumor necrosis factor α, platelet-derived growth factor, and angiotensin II, therefore fail to activate Nox1. Accordingly, the nonphosphorylatable Nox1 mutant T429A failed to produce ROS, whereas the phosphomimetic T429D had an activity similar to the wild-type enzyme. Interestingly, the response to phorbol ester, which leads to a massive activation of the enzyme, was independent of the phosphorylation state of T429, suggesting that the mechanism uncovered by Streeter et al9 is central for physiological signal but can be overcome under certain conditions.

The fact that Nox1 activity is controlled by PKC-mediated phosphorylation on T429 is important for several reasons: The phosphorylation site seems to be unique among the Nox enzymes and the modeling performed by Streeter et al10 suggests that at least on the peptide basis this knowledge can be used for inhibitor development. Indeed, a peptide containing the Nox1 interaction site of Noxa1 has been developed by Ranayhossaini et al10 and turned out to be highly selective and efficient already in the nanomolar range. The study by Streeter et al9 also helps to explain why PKC inhibition is so effective in lowering vascular oxidative stress. In the standard model of Nox activation, AKT and p21-protein activated kinase are discussed as additional upstream activators of Nox enzymes. These kinases can phosphorylate p47phox on multiple serines just as protein kinase C does.4 In contrast to this, the Nox1 T429 phosphorylation seems to be PKC specific. Finally, we still know little about the contribution of the different cytosolic proteins to Nox activation. For the present work, Nox1 is particularly relevant. This protein lacks the autoinhibitory region of p47phox and thus overexpression of Nox1 together with Noxo1 and Noxa1 results in constitutive ROS production. It can be speculated that by T429 phosphorylation of Nox1 this ROS formation is modulated.6,7 The concept is attractive as in situations of oxidative stress, Nox enzymes switch from acute agonist-stimulated ROS production to a continuous mode with high ROS output, which eventually results in oxidative stress. Importantly, Streeter et al9 report that in such conditions, Nox1 is constitutively phosphorylated on T4299 and it was previously realized that inhibition of PKC lowers the chronic Nox1-dependent ROS production present in angiotensin II–induced hypertension10 and diabetes mellitus.10

It is fascinating to experience that within a few years, tremendous progress has been made in identifying molecular mechanisms of Nox biology. Numerous phosphorylation
switches have been identified (Figure 2) and a complex downstream redox-network has been unraveled. Despite all this work, the somewhat teleological question on the true function of Nox enzymes remains open. Of the 7 proteins, only 3 are essential: Nox3 is required for otolite formation in the ear, Duox2 for thyroid hormone production, and Nox2 for host defense by polymorph nuclear neutrophils. Nox1 shares many similarities with Nox2 and potentially arose by gene duplication. The somewhat teleological question on the true function of Nox1 for the human vascular system.

Figure 1. Activation of Nox1 in smooth muscle cells. ? indicates that the presence of the protein is unclear; AngII, angiotensin II; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; TLR, Toll-like receptor; and TNF, tumor necrosis factor.

Figure 2. Control of Nox activity by phosphorylation. Activation of the catalytically active Nox subunit occurs as consequence of its interaction with an activating protein. This process is facilitated by an organizing protein that approximates the Nox protein and its interaction with an activator protein. This process is facilitated by an organizing protein. PKC, protein kinase C; MEK, MAP kinase/ERK kinase; MAPK, mitogen-activated protein kinase; MAPK, MAP kinase.

None.

References

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