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Molecular Mechanism Underlying Activation of Superoxide-Producing NADPH Oxidases: Roles for Their Regulatory Proteins

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SUMMARY: The phagocyte NADPH oxidase is dormant in resting cells but becomes activated during phagocytosis to produce superoxide, a precursor of microbicidal oxidants, thereby playing a crucial role in host defence. The catalytic core of this enzyme comprises the two membranous subunits gp91*phox*/Nox2 and p22*phox*. The oxidase activation requires the small GTPase Rac and the SH3 domain-containing proteins p47*phox* and p67*phox*; they normally exist in the cytoplasm and translocate upon cell stimulation to the membrane. The translocation depends on a stimulus-induced conformational change of p47*phox*, which leads to the SH3 domain-mediated interaction with p22*phox*, a binding required for the gp91*phox*/Nox2-dependent superoxide production. Activation of Nox1, an oxidase that is likely involved in host defence at the colon, requires novel proteins homologous to p47*phox* and p67*phox*, designated Noxo1 and Noxa1, respectively. Noxo1 and Noxa1, both expressed abundantly in the colon, are capable of constitutively activating Nox1. The constitutive activation may be due to the property of Noxo1: in contrast with p47*phox*, Noxo1 seems to normally associate with p22*phox*, which is required for the Nox1 activation. We will also describe the mechanism underlying regulation of the third oxidase Nox3, which exits in fetal kidney and inner ears.

The membrane-integrated protein gp91*phox*, forming a heterodimer with p22*phox*, functions as the catalytic core of the phagocyte NADPH oxidase, which plays a crucial role in host defence. This oxidase is dormant in resting cells, but becomes activated to produce superoxide, a precursor of microbicidal oxidants, by interacting with the adaptor proteins p47*phox* and p67*phox* as well as the small GTPase Rac (Fig. 1) (1,2). In the past few years, several proteins homologous to gp91*phox* were discovered as superoxide-producing NAD(P)H oxidases (the Nox family oxidases); regulatory mechanisms for novel oxidases are currently under intensive investigation. Recent identification of proteins homologous to p47*phox* and p67*phox*, designated as Noxo1 (Nox organizer 1) and Noxa1 (Nox activator 1), respectively (3-5), has shed lights on common and distinct mechanisms underlying activation of Nox oxidases. Here we focus on roles of the novel and classical regulators in activation of Nox1-3.

Regulation of gp91*phox*, the catalytic subunit of the phagocyte NADPH oxidase: Activation of the phagocyte oxidase, i.e. gp91*phox*/Nox2, requires stimulus-induced membrane translocation of cytosolic regulators including the small GTPase Rac and the two specialized cytosolic proteins p67*phox* and p47*phox*, each containing two SH3 domains (Fig. 1). The translocation depends on a stimulus-induced conformational change of p47*phox*, which allows its SH3 domains to interact with p22*phox*; in resting cells p47*phox* is inactive in that the SH3 domains are masked via an intramolecular interaction with the autoinhibitory region (AIR) that exists C-terminal to the domains (2). The binding of p47*phox* to p22*phox* serves as a switch for activation of gp91*phox*/Nox2 (2). Another switch is conversion of Rac to the GTP-bound state, which results in its interaction with p67*phox*. This interaction also plays a crucial role in the phagocyte oxidase activation.

In addition to the classical homologues, Noxo1 and Noxa1 are capable of replacing the corresponding classical homologue in activation of gp91*phox*. p40*phox*, a protein that binds to p67*phox* in the cytoplasm of resting phagocytes (Fig. 1), upregulates the phagocyte oxidase activity by facilitating the translocation of p47*phox* and p67*phox* (1). This stimulatory effect of p40*phox* is totally dependent on its binding to p67*phox*, and p40*phox* facilitates membrane translocation of both p67*phox* and p47*phox*, but not that of Rac. Thus p40*phox* likely participates in activation of the oxidase by regulating membrane recruitment of p67*phox* and p47*phox* (1). In contrast with p67*phox*, its homologue Noxa1 is incapable of interacting with p40*phox*, indicating that p40*phox* is not involved in Noxa1-dependent regulation of Nox oxidases (5).

Regulation of Nox1: Nox1 is abundantly expressed in the colon and probably involved in host defence at this organ. This oxidase can form a complex with p22*phox*, but the complex is in an inactive form by itself: no superoxide production is observed when Nox1 alone (or both Nox1 and p22*phox*) is ectopically expressed in cells (5). On the other hand, Nox1 can be activated when it is coexpressed with Noxo1 and Noxa1, with Noxo1 and p67*phox*, or with p47*phox* and Noxa1 (3-5). The activation by p47*phox* absolutely requires cell stimulation-i.e., stimulation with PMA, a stimulant that is known to potently induce superoxide production by the phagocyte oxidase–, whereas Noxo1-dependent activation of Nox1 is observed even without any stimulants added, suggesting that Noxo1 is normally in an active state (5). Consistent with this, Noxo1, unlike p47*phox*, seems constitutively active in binding to p22*phox* (Fig. 2): the full-length of Noxo1, but not that of p47*phox*, is capable of interacting with p22*phox*, which interaction is likely crucial for Nox1 activation (5). Thus Nox1 appears to be distinctly regulated by Noxo1 and p47*phox*. On the other hand, the interaction of Noxa1 with Rac seems to be involved in activation of Nox1 (Ueno et al., manuscript in preparation).

Regulation of Nox3: Nox3 is expressed in fetal tissues, especially in the kidney, and in the inner ear where it participates in formation of otoconia that is involved in perception of motion and gravity.

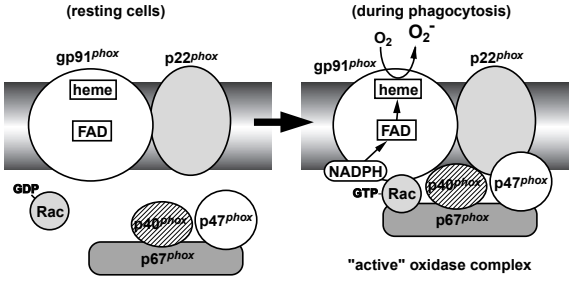

Fig. 1. Activation of gp91*phox*/Nox2.

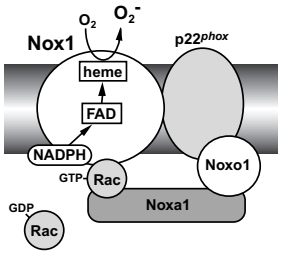

Fig. 2. Activation of Nox1.

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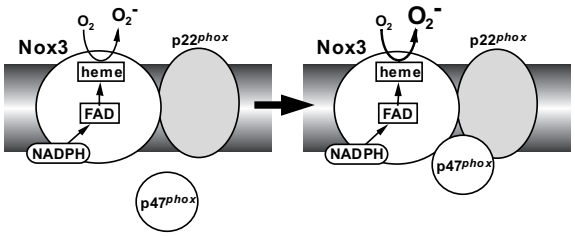

Fig. 3. Regulation of Nox3.

In contrast with the above-mentioned oxidases gp91^{phox}/Nox2 and Nox1, expression of Nox3 alone in various types of cells leads to an NAD(P)H-dependent production of superoxide (Fig. 3): Nox3 produces superoxide without cytosolic regulatory proteins such as p47^{phox}, p67^{phox}, and their homologues, at the level similar to that by Nox1 in the presence of both Noxo1 and Noxa1 (Ueno et al., manuscript in preparation). The Nox3-dependent superoxide production requires the coexpression with p22^{phox} (Ueno et al., manuscript in preparation), indicating that Nox3 also forms a functional complex with p22^{phox} (Fig. 3). PMA enhances the superoxide-producing activity of Nox3 in the presence of p47^{phox}, while PMA is ineffective in the absence of p47^{phox} (6) (Ueno et al., manuscript in preparation). The enhancement with p47^{phox} depends on its interaction with p22^{phox} (Ueno et al., manuscript in preparation), further supporting the involvement of p22^{phox} in the Nox3 system (Fig. 3). On the other hand, Noxa1 fails to facilitate the Nox3 activity (Ueno et al., manuscript in preparation), which is in contrast with the fact that Noxa1 functions as an activator for Nox1 and Nox2 (3-5).

As described here, all the three oxidases Nox1-3 appear to be functionally complexed with p22^{phox}, whereas their superoxide-producing activities are distinctly controlled by SH3 domain-containing regulatory proteins.

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