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Starting-NOX2-Up: Rac unrolls p67^{phox}

Discussion on the molecular mechanisms of phagocyte NADPH oxidase activation.

Phagocytes such as polymorphonuclear neutrophils, eosinophils, monocytes and macrophages express an enzyme dedicated to the generation of reactive oxygen species (ROS), known as the phagocyte NADPH oxidase. It plays a key role in innate immunity and inflammation by producing high quantities of superoxide anion, lighting the wick of ROS generation, a process known as the respiratory burst^{1,2}. The importance of the phagocyte NADPH oxidase in host defense is illustrated in patients with chronic granulomatous disease (CGD), a rare genetic disease caused by mutations in the genes encoding its components^{1,2}. These patients suffer from life threatening infections due to the inability of their phagocytes to produce ROS. The phagocyte NADPH oxidase is an enzymatic complex composed of two membrane proteins, gp91^{phox} or NOX2 and p22^{phox}, along with four cytosolic proteins, p47^{phox}, p67^{phox}, p40^{phox} and the small GTP-binding protein Rac1 (in monocytes and macrophages) or Rac2 (in neutrophils)^{1,2}. NOX2 and p22^{phox} form an heterodimer called cytochrome b558 for its spectral absorption at 558 nm, NOX2 is the catalytic core of the enzyme that binds NADPH and FAD, contains two hemes, and is able to transfer electrons from NADPH to molecular oxygen.

In resting cells, the phagocyte NADPH oxidase components are segregated between membranes and cytosol, thereby keeping the system in a non-activated and harmless state. Thus, to be functional, NOX2 requires the intervention of the cytosolic proteins, each with its specific role. p47^{phox} is known to participate to the organization of the complex, allowing the transfer of p67^{phox} and p40^{phox} to the cytochrome b558, while p67^{phox} is considered as the activating component within the enzymatic complex as it interacts with NOX2³ and initiates the electron transfer by NOX2⁴. p40^{phox} is a stabilizer and an enhancer of NADPH oxidase

activation, especially at the level of the phagosome. Rac-GTP is known to be required for NADPH oxidase activation in a cell-free system and in intact cells by interacting with p67^{phox} and NOX2. However, how p67^{phox} changes from an inactive state to an active state and the role of Rac in this process is still not known. In this issue of the *Journal of Leukocyte Biology*, Bechor et al.⁵ from Edgar Pick's laboratory uncovers the molecular basis through which the cytosolic activating components (p67^{phox} and Rac) "start up" NOX2 enzymatic activity. They report that in the resting state, p67^{phox} is rolled onto itself in a closed conformation, and Rac-GTP unrolls it to unmask a cryptic sequence in p67^{phox}, promoting its binding to NOX2 to start the electron transfer and superoxide anion production (Figure 1).

Human p67^{phox} protein is composed of 526 amino acids (aa), organized into several specific domains. Its N-terminal amino acid sequence has four tetratricopeptide repeats (TPR) (aa 3-36, aa 37-70, aa 71-104 and aa 121-154) that contribute to Rac binding, followed by an activation domain (AD) (aa 199-210), a proline rich region (PRR) (aa 226-236), a first Src homology 3 (SH3A) domain (aa 243-298), a phox and Bem1 (PB1) domain (aa 351-429) and a second SH3 domain (SH3B) (aa 460-514). The X-ray structure of the full-length p67^{phox} is not known; however, the crystal structures of the N-terminal domain (aa 1-203 and aa 1-213) in free form and in a complex with Rac-GTP have been described^{6,7}. Both studies showed that the TPR domains adopt a super-helix fold with a hydrophobic groove ending with a short helix close to the AD and a β hairpin between TPR3 and TPR4, and that hydrogens bonds are formed between the residues in the β -hairpin (aa 105-119) and those in a region C-terminal to the TPR4 (aa 168-186). In addition to these studies, structural analysis of p67^{phox} by Small Angle X-Ray Scattering (SAXS)⁸ and phosphorylation studies⁹ have also suggested the existence of an intra-molecular interaction that might engage the N-terminal SH3 domain (SH3A) and the TPR domains of p67^{phox}.

A previous study from Pick's group¹⁰ proposed that p67^{phox} activates NOX2 following its binding to residues within the sequence 369-383 of the NOX2 dehydrogenase region (DHR). In this new study, Bechor et al., confirm the previous findings and show that Rac-GTP is able to induce a change in the conformation of p67^{phox} that exposes a hidden sequence involved in NOX2 binding in order to stimulate NOX2 activity. They showed that the native p67^{phox} binds weakly to several NOX2 peptides (encompassing aa 342-386), while a p67^{phox} deletion mutant lacking aa 181-193 and p67^{phox} with point mutations (Q115R and K181E), in which the intra-molecular interactions are disrupted because of the loss of the hydrogen bonds mentioned above, were able to strongly bind to the previously identified NOX2 sequence (aa 369-383). This supports the hypothesis that a conformation change within p67^{phox} is required for NOX2 binding. The authors further show that Rac is responsible for this conformational change as a recombinant protein chimera (p67^{phox}-Rac-GTP) exhibited a marked binding to the NOX2 peptide, similar to that of the p67^{phox} mutants. They used size exclusion chromatography to show that the chimera exists in monomeric and polymeric forms, with binding to the NOX2 peptide (aa 369-383) occurring only with the polymers, suggesting that the conformational change within p67^{phox} is intensified in chimera belonging to polymers. These data suggest that Rac-GTP induces a disengagement of the β hairpin from the residues located within the region C-terminal of the TPR to induce a conformational change of p67^{phox} allowing its subsequent interaction with NOX2.

The study by Bechor et al. brings significant new information to the phagocyte oxidase field. The first is that p67^{phox} can exist in closed and open conformations. The closed conformation of p67^{phox} represents a new brake for NOX2 activation and ROS production by the phagocytes, while the open conformation allows p67^{phox} to interact with NOX2 and to stimulate the electron transfer by the activation domain. The second is the identification and mapping of the interacting sequences between NOX2 and p67^{phox}, which could be used to develop new NOX2 inhibitors. The third is that it highlights a novel and important molecular function of Rac in NADPH oxidase

activation, previously thought to be restricted to a transporter role. Thus, this seminal study adds another critical step to our understanding of the phagocyte oxidase activation.

As it is always the case with novel mechanisms, it also raises several questions:

The authors used size exclusion chromatography to demonstrate that the p67^{phox} undergoes a conformational change. Although this approach is suitable, the conformational changes of p67^{phox} will need to be further analyzed using other approaches such as structural and biochemical techniques.

While this study shows a fundamental role of Rac-GTP in promoting p67^{phox} conformational change and its subsequent binding to NOX2, it is probable that it is not the only key player, and p47^{phox} and p40^{phox} could also be important. Indeed, even though both proteins do not have intrinsic catalytic function, cell-free system experiments showed that they can increase the affinity of p67^{phox} to NOX2 and make it more efficient. Furthermore, mutations of p47^{phox} and p40^{phox} genes found in patients with chronic granulomatous disease (CGD) show that they are required for NADPH oxidase activation in intact cells. Thus it will be important to investigate the effects of p47^{phox} and p40^{phox} on the conformation of p67^{phox} in the absence and presence of activating agents such as arachidonic acid or sodium or lithium dodecyl sulfate.

Furthermore, p67^{phox} is phosphorylated on different sites located in the N-terminal and C-terminal portions of the protein, although only one site (Thr233) has been clearly identified so far. Thus, p67^{phox} phosphorylation could also modulate the conformational changes and should be further investigated in the light of the new findings by Bechor et al..

Another unanswered question is how the p67^{phox}-activation domain (AD) initiates electrons transfer within NOX2 once it is bound to NOX2. In this process, Rac-GTP might also play a key role as it is noticeable in this study, that p67^{phox}-Rac-GDP chimera also binds significantly to the NOX2 peptide, but only weakly induces NADPH oxidase activity in a cell-free system in contrast to the p67^{phox}-Rac-GTP chimera. Therefore, in addition to its ability to induce p67^{phox}

conformational change that allows binding to NOX2, Rac-GTP might also play a key role in allowing the AD domain to become functional.

The last point we would like to discuss relates to whether this new molecular mechanism applies to the activation of the NOX2 homologues. NOX1 is the closest homologue of NOX2 mainly expressed in colon epithelial cells. NOX1 is regulated by the cytosolic components NOXA1 and NOXO1, homologous to p67^{phox} and p47^{phox} respectively and by Rac1. Human NOXA1 is composed of 476 amino acids (aa), and like p67^{phox}, its N-terminal amino acid sequence has four TPRs (aa 6-155) that contribute to Rac binding, followed by the AD (aa 100-114) and a PB1 domain (aa 299-392) and only one SH3 domain (aa 401-460). NOX1 is constitutively active and its activity can be enhanced by inflammatory mediators (TNF α , IL-17, LPS...) and chemical agents such as phorbol myristate acetate (PMA). It would be of interest to determine whether NOXA1 is in a closed or open conformation and whether Rac-GTP induces conformational changes. It is worth noting that K181 and Q115 within p67^{phox} are not present in NOXA1; instead, R184 and its partner G113 are present, and this bond could be sufficient to maintain NOXA1 in a closed conformation.

In summary, the work by Bechor et al. is a major advance in our understanding of ROS production by phagocytes as provides important findings on the molecular mechanism of the phagocyte NADPH oxidase activation. It also opens up new avenues for a better understanding of NOXs dysregulation in several immunological and inflammatory diseases and raises intriguing questions for NOXs scientific field to be explored.

FIGURE 1 A proposed model for a closed and open conformation of p67^{phox} and how Rac-GTP induces p67^{phox}-conformational change and interaction with NOX2. P67^{phox} protein is composed of four tetratricopeptide repeats (TPR) (aa 3-36, aa 37-70, aa 71-104 and aa 121-154), followed by an amino acid sequence contains an activation domain (AD) (aa 199-210), a poly-proline region (PRR) (aa 226-236), an SH3 domains (aa 243-298(SH3A)), a PB1 (phox and Bem1 domain) domain (aa 351-429) and a second SH3 domain (aa 460-514(SH3B)). In the resting state, p67^{phox} is in closed conformation in which the NOX2 binding sequence (yellow circle) is hidden and thus inaccessible to NOX2. This closed conformation is maintained by intramolecular bond(s) (Red dashed line) between residues in the β hairpin (G113 and Q115) and residues of the C-terminal to the TPR domains (R184 and K181, respectively). Binding of Rac to the TPR uncouples these intramolecular bonds to expose the NOX2-binding sequence of p67^{phox} which interacts with aa 375-383 of the NOX2 DHR in an open conformation.

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REFERENCES

1. Nauseef WM. How human neutrophils kill and degrade microbes: an integrated view. *Immunol Rev.* 2007 Oct;219:88-102.
2. El-Benna J, Dang PMC, Hurtado-Nedelec M, Marie JC, Gougerot-Pocidallo MA. Priming of the neutrophil respiratory burst : Role in host defense and inflammation. *Immunol Rev.* 2016 Sep;273(1):180-193.
3. Dang PM-C, Cross AR, Babior BM. Assembly of the neutrophil respiratory burst oxidase: a direct interaction between p67phox and cytochrome b558. *Proc Natl Acad Sci USA.* 2001;98:3001-3005.
4. Han C-H, Freeman JLR, Lee TH, Motalebi SA, Lambeth JD. Regulation of the neutrophil respiratory burst oxidase – identification of an activation domain in p67phox. *J Biol Chem.* 1998;273:16663-16668.
5. Bechor E, Zahavi A, Berdichevsky Y, and Edgar Pick. The molecular basis of Rac-GTP action – Promoting binding of p67^{phox} to Nox2 by disengaging the β hairpin from downstream residues. *J Leuk Biol* 2021(This issue).
6. Lapouge K, Smith SM, Walker, PA, Gamblin SJ, Smerdon SJ, Rittinger K. Structure of the TPR domain of p67phox in complex with Rac-GTP. *Molec Cell.* 2000;6:899-907.
7. Grizot S, Fieschi F, Dagher M-C, Pebay-Peyroula E. The active N-terminal region of p67phox : Structure at 1.8 Å resolution and biochemical characterizations of the A128V mutant implicated in chronic granulomatous disease *J Biol Chem.* 2001;276:21627-21631.
8. Durand D, Vivès C, Cannella D, Pérez J, Pebay-Peyroula E, Vachette P, Fiesch F. NADPH oxidase activator p67(phox) behaves in solution as a multidomain protein with semi-flexible linkers. *J Struct Biol.* 2010 Jan;169(1):45-53.
9. Dang PM-C, Morel F, Gougerot-Pocidallo M-A, El Benna J. Phosphorylation of the NADPH oxidase component p67phox by ERK2 and P38MAPK: Selectivity of phosphorylated sites and existence of an intramolecular regulatory domain in the tetratricopeptide-rich region. *Biochemistry.* 2003;42:4520-4526.
10. Bechor E, Zahavi A, Amichay M, Fradin T, Federman A, Berdichevsky Y, Pick E. p67phox binds to a newly identified site in Nox2 following the disengagement of an intramolecular bond – Canaan sighted? *J Leukoc Biol.* 2020;107:509-528.