RHO GTPases Activate NADPH Oxidases

Ballmer-Hofer, K., Berger, P., Garapati, P V., Jupe, S., Kuijpers, TW., Nüsse, O., Orlic-Milacic, M., Rivero Crespo, F., Shamovsky, V., Welsh, M.

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18/11/2022
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 82

This document contains 1 pathway and 14 reactions (see Table of Contents)
NADPH oxidases (NOX) are membrane-associated enzymatic complexes that use NADPH as an electron donor to reduce oxygen and produce superoxide (O2-) that serves as a secondary messenger (Brown and Griendling 2009).

NOX2 complex consists of CYBB (NOX2), CYBA (p22phox), NCF1 (p47phox), NCF2 (p67phox) and NCF4 (p40ohox). RAC1:GTP binds NOX2 complex in response to VEGF signaling by directly interacting with CYBB and NCF2, leading to enhancement of VEGF-signaling through VEGF receptor VEGFR2, which plays a role in angiogenesis (Ushio-Fukai et al. 2002, Bedard and Krause 2007). RAC2:GTP can also activate the NOX2 complex by binding to CYBB and NCF2, leading to production of superoxide in phagosomes of neutrophils which is necessary for the microbicidal activity of neutrophils (Knaus et al. 1991, Roberts et al. 1999, Kim and Dinauer 2001, Jyoti et al. 2014).

NOX1 complex (composed of NOX1, NOXA1, NOXO1 and CYBA) and NOX3 complex (composed of NOX3, CYBA, NCF1 amd NCF2 or NOXA1) can also be activated by binding to RAC1:GTP to produce superoxide (Cheng et al. 2006, Miyano et al. 2006, Ueyama et al. 2006).

Literature references


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NADPH oxidase 2 (NOX2) complex binds RAC1

Location: RHO GTPases Activate NADPH Oxidases

Stable identifier: R-HSA-5218827

Type: binding

Compartments: plasma membrane

NADPH oxidase (NOX) proteins are membrane-associated, multiunit enzymes that catalyze the reduction of oxygen using NADPH as an electron donor. NOX proteins produce superoxide (O2.-) via a single electron reduction (Brown & Griendling 2009). Superoxide molecules function as second messengers to stimulate diverse redox signaling pathways linked to various functions including angiogenesis. VEGF specifically stimulates superoxide production via RAC1 dependent activation of NOX2 complex. VEGF rapidly activates RAC1 and promotes translocation of RAC1 from cytosol to the membrane. At the membrane RAC1 interacts with the NOX enzyme complex via a direct interaction with NOX2 (gp91phox or CYBB) followed by subsequent interaction with the NCF2 (Neutrophil cytosol factor 2) or p67phox subunit and this makes the complex active (Bedard & Krause 2007). O2.- derived from Rac1-dependent NOX2 are involved in oxidation and inactivation of protein tyrosine phosphatases (PTPs) which negatively regulate VEGFR2, thereby enhancing VEGFR2 autophosphorylation, and subsequent redox signaling linked to angiogenic responses such as endothelial cell proliferation and migration (Ushio-Fukai 2006, 2007).

Followed by: NADPH oxidase 2 generates superoxide from oxygen

Literature references


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The activated NOX2 complex generates superoxide (O₂⁻) by transferring an electron from NADPH in the cytosol to oxygen on the luminal or extracellular space (Bedard & Krause 2007).

**Preceded by:** NADPH oxidase 2 (NOX2) complex binds RAC1

**Literature references**

RAC2:GTP binds NOX2 complex

**Location:** RHO GTPases Activate NADPH Oxidases

**Stable identifier:** R-HSA-5668605

**Type:** binding

**Compartments:** phagocytic vesicle membrane

In neutrophils, RAC2 regulates NADPH oxidase NOX2 complex (Knaus et al. 1991, Kim et al. 2001) which consists of CYBB (NOX2), CYBA (p22phox), NCF1 (p47phox), NCF2 (p67phox) and NCF4 (p40phox). GTP-bound RAC2 binds to a conserved region of CYBB and tetratricopeptide repeats of NCF2 (Koga et al. 1999, Lapouge et al. 2000, Kao et al. 2008).

**Followed by:** S100A8:S100A9:AA:Ca(2+) binds NOX2 complex, Production of phagocyte oxygen radicals by NOX2 complex bound to RAC2:GTP

**Literature references**


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Production of phagocyte oxygen radicals by NOX2 complex bound to RAC2:GTP

**Location:** RHO GTPases Activate NADPH Oxidases

**Stable identifier:** R-HSA-5668629

**Type:** transition

**Compartments:** phagocytic vesicle, phagocytic vesicle membrane, phagolysosome, cytosol

RAC2:GTP-bound NOX2 complex, consisting of CYBB (NOX2), CYBA (p22phox), NCF1 (p47phox), NCF2 (p67phox) and NCF4 (p40phox), acts as an NADPH oxidase to produce superoxide anion O2- in phagosomes of neutrophils, enabling microbicidal activity of neutrophils (Knaus et al. 1991, Kim et al. 2001, Kao et al. 2008, Anderson et al. 2010, Jyoti et al. 2014). Rac2 knockout mice have dramatically reduced NADPH oxidase activity (Roberts et al. 1999). Phosphorylation of NOX2 complex components NCF1 (el Benna et al. 1994), NCF2 (Zhao et al. 2005) and NCF4 (Bouin et al. 1998) contributes to the activation of the phagosomal NADPH oxidase.

**Preceded by:** RAC2:GTP binds NOX2 complex

**Literature references**

Kim, C., Zhen, L., Kapur, R., Dinauer, MC., Petryniak, B., Williams, DA. et al. (1999). Deficiency of the hematopoietic cell-specific Rho family GTPase Rac2 is characterized by abnormalities in neutrophil function and host defense. *Immunity, 10*, 183-96.


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Activated RAC1 (RAC1:GTP) binds NADPH oxidase NOX1 complex composed of NOX1, NOXA1, NOXO1 and CYBA (p22phox). RAC1 directly interacts with a conserved region in NOX1 and with tetratricopeptide repeats in NOXA1 (Takeya et al. 2003, Park et al. 2006, Cheng et al. 2006, Miyano et al. 2006, Kao et al. 2008)

**Literature references**


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The activity of the non-phagocytic NADPH oxidase 1 (NOX1) complex, composed of NOX1, NOXA1, NOXO1 and CYBA, is greatly enhanced upon RAC1:GTP binding, resulting in production of the superoxide O2- which can serve as a second messenger (Takeya et al. 2003, Miyano et al. 2006, Park et al. 2006, Cheng et al. 2006).

**Literature references**


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Activated RAC1 (RAC1:GTP) binds to the NADPH oxidase NOX3 complex, consisting of NOX3, CYBA (p22phox), NCF1 (p47phox) and NCF2 (p67phox) or NOXA1. RAC1 directly interacts with a conserved region of NOX3 and with tetratricopeptide repeats of NCF2 or NOXA1 (Ueyama et al. 2006, Miyano and Sumimoto 2007, Kao et al. 2008).

Followed by: NOX3 complex:RAC1:GTP generates superoxide from oxygen

Literature references


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NOX3 complex:RAC1:GTP generates superoxide from oxygen

**Location:** RHO GTPases Activate NADPH Oxidases

**Stable identifier:** R-HSA-5668731

**Type:** transition

**Compartments:** plasma membrane, extracellular region, cytosol

While NOX3:CYBA complex has constitutive NADPH oxidase activity, the presence of NCF1, NCF2 or NOXA1 and RAC1:GTP enhances the production of superoxide O2- by the NOX3:CYBA complex. When NCF1 is replaced with NOXO1, RAC1:GTP becomes dispensible for the full activation of the NOX3 complex (Ueno et al. 2005, Ueyama et al. 2006, Miyano and Sumimoto 2007, Kao et al. 2008)

**Preceded by:** RAC1:GTP binds NOX3 complex

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1-phosphatidyl-1D-myo-inositol 3-phosphate (PI3P) is generated largely by the Class III PI3 kinase Phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3, Vps34), which is found in intracellular membrane complexes with Phosphoinositide 3-kinase regulatory subunit 4 (PIK3R4, Vps150), necessary for catalytic activity, localization and stability (Florey & Overholtzer 2012, Raiborg et al. 2013). These core subunits are frequently associated with other partners such as Rab5, Beclin-1 and UVRAG.

PI3P strongly upregulates phagosomal NADPH oxidase activity (Hawkins et al. 2010). This effect is mediated by PI3P binding to the PX domain of Neutrophil cytosol factor 4 (NCF4, p40phox), a component of the NOX2 complex. PI3P controls ROS production by regulating the presence of NCF4 and NCF2 (p67phox) at the phagosomal membrane (Song ZM et al. 2017).

**Literature references**

MAPK1 or MAPK3 phosphorylates NCF1 at Ser345

**Location:** RHO GTPases Activate NADPH Oxidases

**Stable identifier:** R-HSA-9626832

**Type:** transition

**Compartments:** cytosol

In resting cells, the NADPH oxidase components, NCF1 (p47phox), NCF2 (p67phox), and NCF4 (p40phox) are located in the cytosol where they associate in a trimer complex with a 1:1:1 stoichiometry through specific domains (Groemping Y & Rittinger K 2005; El-Benna J et al. 2005; Park JW et al. 1994; Lapouge K et al. 2002; El-Benna J et al. 2016). However, NCF1 may also exist separately from the trimer (El-Benna J et al. 2016). In the resting state, two SH3 domains of NCF1 (p47phox) bind the auto-inhibitory region (AIR; amino acids 292-340) to keep NCF1 in a closed auto-inhibited state, preventing its binding to p22phox and therefore NOX2 activation (Groemping Y et al. 2003; Yuzawa S et al. 2004; El-Benna J et al. 2016). Priming of neutrophils by several agents such as GM-CSF, TNFa, PAF, LPS and CL097, a TLR7/8 agonist, induces partial phosphorylation of NCF1 (Makni-Maalej K et al. 2015; Dang PM et al. 1999; Dewas C et al. 2003; DeLeo FR et al. 1998). Mass spectrometry analysis of NCF1 identified Ser345 as the phosphorylated site in neutrophils primed by TNFa and GM-CSF, and site-directed mutagenesis of Ser345 and use of a competitive inhibitory peptide containing the Ser345 sequence have demonstrated that this step is critical for the priming of ROS production in human neutrophils (Dang PMC et al. 2006). Further, inhibitors of the MAPK1 and MAPK3 (ERK1/2) pathway abrogated GM-CSF-induced phosphorylation of Ser345 (Dang PMC et al. 2006).

**Followed by:** PIN1 binds p-S345-NCF1

**Literature references**


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MAPK11 or MAPK14 phosphorylates NCF1 at Ser345

**Location:** RHO GTPases Activate NADPH Oxidases

**Stable identifier:** R-HSA-9626880

**Type:** transition

**Compartments:** cytosol

In resting cells, the neutrophil cytosolic factor 1 (NCF1, also known as p47phox), NCF2 (p67phox), and NCF4 (p40phox) are located in the cytosol where they associate in a trimer complex with a 1:1:1 stoichiometry through specific domains (Groemping Y & Rittinger K 2005; El-Benna J et al. 2005; Park JW et al. 1994; Lapouge K et al. 2002; El-Benna J et al. 2016). However, NCF1 may also exist separately from the trimer (El-Benna J et al. 2016). In the resting state, two SH3 domains of NCF1 (p47phox) bind the auto-inhibitory region (AIR; amino acids 292-340) to keep NCF1 in a closed auto-inhibited state, preventing its binding to p22phox and therefore NOX2 activation (Groemping Y et al. 2003; Yuzawa S et al. 2004; El-Benna J et al. 2016). Priming of neutrophils by several agents such as GM-CSF, TNFα, PAF, LPS and CL097, a TLR7/8 agonist, induces partial phosphorylation of NCF1 (Makni-Maalej K et al. 2015; Dang PM et al. 1999; Dewas C et al. 2003; DeLeo FR et al. 1998). Mass spectrometry analysis of NCF1 identified Ser345 as the phosphorylated site in human neutrophils primed by TNFα and GM-CSF (Dang PMC et al. 2006). Site-directed mutagenesis of Ser345 and use of a competitive inhibitory peptide containing the Ser345 sequence have demonstrated that this step is critical for the priming of ROS production in human neutrophils (Dang PMC et al. 2006). Further, inhibitors of the p38 MAPK abrogated TNF-alpha- and TLR8 agonist-induced phosphorylation of Ser345 (Dang PMC et al. 2006; Makni-Maalek K et al. 2015).

**Followed by:** PIN1 binds p-S345-NCF1

**Literature references**


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https://reactome.org
PIN1 binds p-S345-NCF1

**Location:** RHO GTPases Activate NADPH Oxidases

**Stable identifier:** R-HSA-9626816

**Type:** transition

**Compartments:** cytosol

Priming agents such as tumor necrosis factor-α (TNFα) and toll like receptor 7 (TLR7)/TLR8 agonists induced the activation of the peptidyl-prolyl cis/trans isomerase PIN1 in human neutrophils (Boussetta T et al. 2010; Makni-Maalej K et al. 2015). PIN1 is an enzyme that binds to phosphorylated Ser-Pro or Thr-Pro sequences, and subsequently catalyzes their conformational changes (Liou YC et al. 2011). In intact neutrophils, PIN1 was found to bind to the neutrophil cytosol factor 1 (NCF1 or p47phox) via the phosphorylated residue of Ser345 (Boussetta T et al. 2010). PIN1 then catalyzed a conformational change of NCF1 that facilitated subsequent phosphorylation of the protein on other sites by protein kinase C (PKC) (Boussetta T et al. 2010; El-Benna J et al. 2016). Extensive phosphorylation of the subunit NCF1 (p47phox) occurs during the activation of the NADPH oxidase (NOX2) in intact cells.

**Preceded by:** MAPK11 or MAPK14 phosphorylates NCF1 at Ser345, MAPK1 or MAPK3 phosphorylates NCF1 at Ser345

**Followed by:** PKC phosphorylates NCF1

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[https://reactome.org](https://reactome.org)
Neutrophil cytosolic factor 1 (NCF1, also known as p47phox) is a component of the NADPH oxidase (NOX2) complex, which consists of six subunits (Groemping Y et al. 2003; El-Benna J et al. 2005). Two of these subunits, p22phox and gp91phox, are integral membrane proteins and form a heterodimeric flavocytochrome that constitutes the catalytic core of the enzyme. The remaining oxidase components reside in the cytosol and include the small GTPase Rac, as well as a complex of NCF4 (p40phox), NCF1, and NCF2 (p67phox) (Groemping Y et al. 2003; El-Benna J et al. 2005). In the resting state, the interaction of NCF1 (p47phox) with p22phox, and thereby translocation and NADPH oxidase activation, is prevented by an auto-inhibited conformation of NCF1 (Groemping Y et al. 2003; Yuzawa S et al. 2004). This is believed to arise from an intramolecular interaction of the SH3 domains with the C-terminal auto-inhibitory region (AIR) (amino acids 292-340) of NCF1 to keep the protein 'locked' (Groemping Y et al. 2003; El Benna J et al. 2016). Priming induced by TNF-α or GM-CSF induces NCF1 phosphorylation on Ser345, activation of the proline isomerase PIN1, which binds to NCF1 to induce conformational changes (Boussetta T et al. 2010). This process facilitates extensive phosphorylation of NCF1 by PKC on other sites and induces full opening of NCF1 (Boussetta T et al. 2010). Phosphorylation studies showed that p47phox is phosphorylated on serines located between Ser303 and Ser379 (El Benna J et al. 1994; 2009). Most of these sites correspond to PKC consensus phosphorylation sites, and PKCa, -β, -δ and -ζ were all shown to phosphorylate NCF1 (p47phox) in vitro or in human neutrophil-like HL-60 cells (Dang PM et al. 2001; Fontayne A et al. 2002; Belambi SA et al. 2018). In vitro studies also showed that phosphorylation of p47phox induced its binding to the proline rich region (PRR) of p22phox and enhanced the binding of NCF2 (p67phox) to gp91phox (Fontayne A et al. 2002; Dang PMC et al. 2002; Boussetta T et al. 2010).

The Reactome event depicts the PKC-mediated phosphorylation of NCF1 on Ser303, Ser304, Ser320, Ser328, Ser348. However, NCF1 becomes phosphorylated by PKCs on multiple sites and the number of sites is not defined.
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Ca(2+) flux across the phagosomal membrane influences NADPH oxidase activity and ROS production. Phagocytic engagement of Fc gamma receptor (FcγR) or complement receptor 3 (CR3) activate phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), leading to the formation of PI(3,4,5)P3. This phospholipid participates in the activation of phospholipase γ C (PLCγ) and phospholipase D (PLD)-mediated downstream signaling pathways. The generation of IP3 by PLCγ triggers Ca(2+) release from intracellular stores (endoplasmic reticulum, ER) via the opening of IP3 receptors (IP3-R). PLD is involved in the process of sphingosine kinase-produced sphingosine 1-phosphate (S1P), leading to the depletion of intracellular Ca(2+) stores. The emptying of intracellular Ca2+ stores induces the activation of the Ca(2+) sensor stromal interaction molecule-1 (STIM1), which, in turn, activates calcium release-activated calcium channel protein 1 (ORAI1) at the plasma membrane and extracellular Ca(2+) entry. The resulting elevation of Ca(2+) mediates the recruitment of the cytosolic Ca(2+)-activated regulators S100A8 (also know as migration inhibitory factor-related proteins 8 (MRP8)) and S100A9 (MRP14) to the phagosomal membrane (Berthier S et al. 2003, 2012; Steinckwich N et al. 2011; Bréchard S et al. 2013). The translocation of S100A8:S100A9 allows the transfer of S100A9-binding arachidonic acid (AA) to cytochrome b558, favoring the conformational change of cytochrome b558 and promoting intraphagosomal NADPH oxidase activation and ROS production (Berthier S et al. 2003, 2012; Doussiere J et L. 2002; Kerkhoff C et al. 2005; Steinckwich N et al. 2011; Bréchard S et al. 2013 ). S100A8 & S100A9 exist mainly as a S100A8:S100A9 heterodimer which is termed calprotectin based on its role in innate immunity (Korndorfer IP et al. 2007). Ca(2+) is also known to stimulate formation of higher order oligomers of S100 proteins, including S100A8/S100A9 tetramers (Leukert N et al. 2006; Korndörfer IP et al. 2007). In addition, calprotectin has been shown to inhibit bacterial growth through chelation of extracellular manganese Mn(2+), zinc Zn(2+) and possibly iron Fe(2+) and thus restricting metal-ion availability during infection (Damo SM et al. 2013; Hayden JA et al. 2013; Brophy MB et al. 2013; Gagnon DM et al. 2015).

**Preceded by:** RAC2:GTP binds NOX2 complex

**Literature references**


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<th>Date</th>
<th>Action</th>
<th>Author/Reviewer</th>
</tr>
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<tr>
<td>2018-10-30</td>
<td>Authored, Edited</td>
<td>Shamovsky, V.</td>
</tr>
<tr>
<td>2018-11-07</td>
<td>Reviewed</td>
<td>Nüsse, O.</td>
</tr>
</tbody>
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- Introduction
- RHO GTPases Activate NADPH Oxidases
  - NADPH oxidase 2 (NOX2) complex binds RAC1
  - NADPH oxidase 2 generates superoxide from oxygen
  - RAC2:GTP binds NOX2 complex
  - Production of phagocyte oxygen radicals by NOX2 complex bound to RAC2:GTP
  - RAC1:GTP binds NOX1 complex
  - NOX1 complex:RAC1:GTP generates superoxide from oxygen
  - RAC1:GTP binds NOX3 complex
  - NOX3 complex:RAC1:GTP generates superoxide from oxygen
  - PIK3C3:PIK3R4 phosphorylates PI to PI3P
  - MAPK1 or MAPK3 phosphorylates NCF1 at Ser345
  - MAPK11 or MAPK14 phosphorylates NCF1 at Ser345
  - PIN1 binds p-S345-NCF1
  - PKC phosphorylates NCF1
  - S100A8:S100A9:AA:Ca(2+) binds NOX2 complex

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