
The following resources related to this article are available online at <http://stke.sciencemag.org>.
This information is current as of 4 April 2007.

- Article Tools** Visit the online version of this article to access the personalization and article tools:
<http://stke.sciencemag.org/cgi/content/full/sigtrans;2006/349/re8>
- Related Content** The editors suggest related resources on *Science's* sites:
<http://stke.sciencemag.org/cgi/content/abstract/sigtrans;2006/332/eg6>
- References** This article cites 57 articles, 35 of which can be accessed for free:
<http://stke.sciencemag.org/cgi/content/full/sigtrans;2006/349/re8#otherarticles>
- Glossary** Look up definitions for abbreviations and terms found in this article:
<http://stke.sciencemag.org/glossary/>
- Permissions** Obtain information about reproducing this article:
<http://www.sciencemag.org/about/permissions.dtl>

Science's Signal Transduction Knowledge Environment (ISSN 1525-8882) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright © 2006 by the American Association for the Advancement of Science; all rights reserved. The title Science's Signal Transduction Knowledge Environment is a registered trademark of AAAS.

Localizing NADPH Oxidase–Derived ROS

Masuko Ushio-Fukai

(Published 22 August 2006)

Reactive oxygen species (ROS) function as signaling molecules to mediate various biological responses, including cell migration, growth, and gene expression. ROS are diffusible and short-lived molecules. Thus, localizing the ROS signal at the specific subcellular compartment is essential for activating redox signaling events after receptor activation. NADPH (nicotinamide adenine dinucleotide phosphate) oxidase is one of the major sources of ROS in vasculature; it consists of a catalytic subunit (Nox1, Nox2, Nox3, Nox4, or Nox5), p22phox, p47phox, p67phox, and the small guanosine triphosphatase Rac1. Targeting of NADPH oxidase to focal complexes in lamellipodia and membrane ruffles through the interaction of p47phox with the scaffold proteins TRAF4 and WAVE1 provides a mechanism for achieving localized ROS production, which is required for directed cell migration. ROS are believed to inactivate protein tyrosine phosphatases, which concentrate in specific subcellular compartments, thereby establishing a positive feedback system that activates redox signaling pathways to promote cell movement. Additionally, ROS production may be localized through interactions of NADPH oxidase with signaling platforms associated with lipid rafts and caveolae, as well as with endosomes. There is also evidence that NADPH oxidase is found in the nucleus, indicating its involvement in redox-responsive gene expression. This review focuses on targeting of NADPH oxidase to discrete subcellular compartments as a mechanism of localizing ROS and activation of downstream redox signaling events that mediate various cell functions.

Introduction

Although excess reactive oxygen species (ROS) are toxic, physiological concentrations of ROS may function as signaling molecules to mediate various responses, including cell migration and growth (1, 2). ROS may be produced in response to receptor activation. However, given that ROS are diffusible and short-lived, localizing the ROS signal at the precise subcellular compartment after receptor activation is essential for stimulation of specific redox signaling. NADPH oxidases are the major sources of ROS in vasculature. In phagocytic cells, NADPH (nicotinamide adenine dinucleotide phosphate) oxidases consist of the membrane-bound cytochrome b558 comprising the catalytic gp91phox and the p22phox subunits, as well as cytosolic components including p47phox, p67phox, and the small Rho guanosine triphosphatase (GTPase) Rac1 (3). Several homologs of gp91phox (also termed Nox2)—Nox1, Nox3, Nox4, and

Nox5—have been identified in nonphagocytic cells (4). Although the evidence is strong that ROS generated by NADPH oxidases participate in signal transduction, the mechanisms by which receptors activate NADPH oxidase and regulate ROS production are poorly understood. NADPH oxidases are now recognized to have specific subcellular localizations. This targeting is required for localized ROS production and activation of specific redox signaling pathways that are temporally and spatially coupled to oxidant-sensitive proteins that mediate various cell functions.

Localizing the ROS Signal for Directional Migration

Migrating cells create transitory integrin-containing structures with tyrosine-phosphorylated proteins termed focal complexes (5). Leading-edge focal complexes bear the strongest tensile forces in migrating cells, and focal complex turnover is necessary for the remodeling of lamellipodia to promote membrane ruffling. The Rho GTPases Rac1 and Cdc42 are involved in focal complex formation within lamellipodia and filopodia, respectively, whereas RhoA facilitates the maturation of focal complexes into stable focal adhesions (5). Rac1 directs the tyrosine kinase Src to lamellipodia to phosphorylate focal complex proteins, including Pyk2, thus promoting the turnover of focal complexes (6, 7). Directed cell migration is a highly localized process involving the generation of spatially and temporally restricted signaling molecules, including Rac1 (8) and phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P₃] (9), which is the product of phosphatidylinositol 3-kinase (PI3K), at the site of the new leading edge. Endothelial cell (EC) migration is a key event for tissue repair in response to injury, angiogenesis, and wound healing. In ECs, Rac1- and Nox2-dependent NADPH oxidase plays an important role in cell migration (10–13). The PI3K–Rac pathway is involved in the production of ROS that accumulate at the membrane ruffles (14), and this locally produced ROS is required for cytoskeletal reorganization and directed cell migration (15, 16). In addition to Rac1, the p47phox and Nox2 subunits of NADPH oxidase are also targeted to the focal complexes in lamellipodia and to membrane ruffles (16–18). Thus, Rac1-dependent NADPH oxidase activation and lamellipodial dynamics are likely to be spatially and functionally coordinated at the leading edge to promote directional cell migration.

Although the details of how NADPH oxidase and Rac1 are coordinately regulated remain unknown, the molecular players that recruit NADPH oxidase to focal complexes are beginning to be discovered. Wu *et al.* (18) identified targeting molecules that may specify the site of ROS production during cell migration. In ECs, the p47phox subunit of NADPH oxidase binds to the orphan adaptor TRAF4, which in turn binds to the focal contact scaffold Hic-5, thereby targeting p47phox to the focal complexes. Thus, there is local activation of NADPH oxidase and ROS production. Cell migration is blocked by knockdown of TRAF4 or Hic-5 using short interfering RNA (siRNA), by disruption of the TRAF4–Hic-5 complex, or by inhibition of ROS with antioxidants or of NADPH oxidase with a mutant form of p67phox;

Department of Pharmacology and Center for Lung and Vascular Biology, University of Illinois College of Medicine, Chicago, IL 60612, USA. E-mail: mfukai@uic.edu

these findings indicate that localized ROS signal at lamellipodial focal complexes through formation of a p47phox–TRAF4–Hic-5 complex is important for cell migration (Fig. 1).

It is currently believed that H₂O₂ functions as a signaling molecule by reversible oxidative inactivation of protein tyrosine phosphatases (PTPs) at cysteines in the catalytic site (19, 20) and by altering protein structure by oxidation of reactive thiols

and function upstream or downstream of the Rho GTPases. PTP-PEST is one PTP that is localized to focal complexes through direct binding to paxillin and Hic-5. PTP-PEST inhibits Rac1 activity and phosphorylation of Pyk2 and Src (25), and thus inhibits membrane ruffling. Furthermore, PTP-PEST blocks focal complex turnover and polarized cell movement (25). Myristoylated TRAF4 and p47phox target to nascent focal

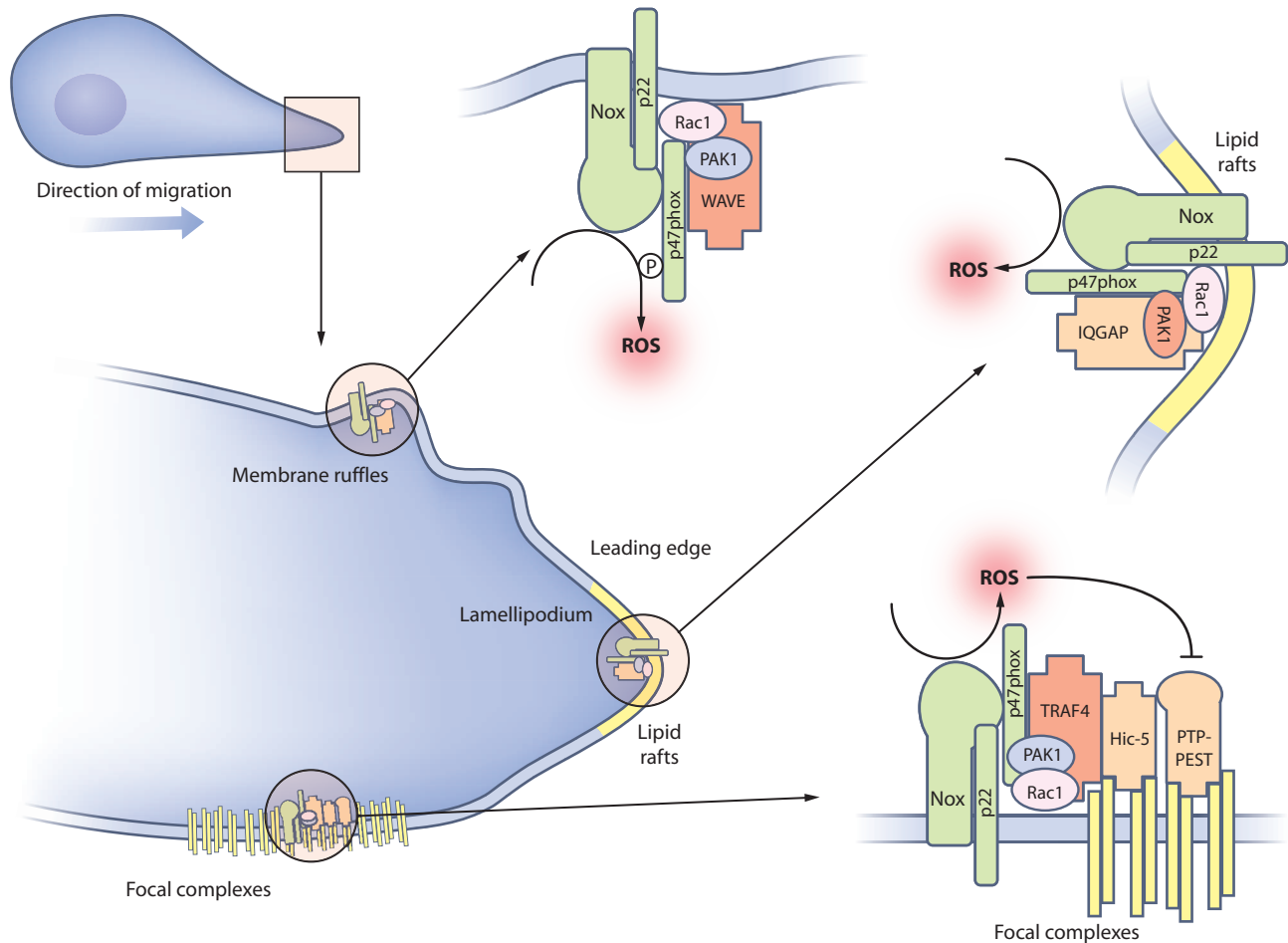


Fig. 1. Localizing the ROS signal for directed cell migration. Targeting p47phox to the focal complexes is mediated through formation of p47phox-TRAF4-Hic5 complex, whereas p47phox targeting to the membrane ruffles is mediated through its direct binding to WAVE1, which contributes to localized ROS production and directed EC migration. p47phox also associates with active Rac1 and its effector kinase PAK1, thereby phosphorylating p47phox and activating NADPH oxidase. Localized ROS production induces oxidative inactivation of protein tyrosine phosphatase PTP-PEST, which localizes to focal complexes through direct binding to Hic-5. Inactivation of PTP-PEST activates Rac1 and PAK1, which phosphorylates p47phox, creating a positive feedback loop that facilitates localized NADPH oxidase-dependent ROS production. Targeting Rac1 to the lamellar leading edge is dependent on lipid rafts, which are also the site of integrin-mediated adhesions. IQGAP1, a downstream target of active Rac1, functions as a scaffold protein to target Nox2 to the leading edge to localize ROS production, which may contribute to directed EC migration.

(21). The lipid phosphatase PTEN, which along with PI3K controls PI(3,4,5)P₃ abundance, is an important target of f (22). Because the local concentration of PI(3,4,5)P₃ is an important factor determining the sites of new actin-filled projections (23, 24), localized production of H₂O₂ might be responsible for controlling the temporal and spatial concentrations of PI(3,4,5)P₃ that activate downstream signaling events (22).

Many PTPs concentrate in specific subcellular compart-

ment-like structures, which induces local oxidative inactivation of PTP-PEST (18) (Fig. 1). Inhibition of PTP-PEST in turn activates Rac1 and its effector kinase PAK1, thereby promoting p47phox phosphorylation and creating a positive feedback loop that facilitates NADPH oxidase activation, local ROS production, and Rac1 activation. Furthermore, targeted inactivation of PTP-PEST by localized ROS production through formation of TRAF4-Rac1-PAK1 complexes facilitates redox-sensitive acti-

vation of Src and Pyk2 at focal complexes. These responses, in turn, promote focal complex turnover and membrane ruffle formation as well as cell migration. It should be noted that during chemotaxis, PTEN is specifically localized to the back of the cell membrane, whereas its substrate PI(3,4,5)P₃ is concentrated at the front, leading edge (26). These results suggest that PTEN regulates directed cell migration by targeting to the opposite site of cells from leading edge where NADPH oxidase-derived ROS and PI(3,4,5)P₃ accumulate, thereby sensing and amplifying the PI(3,4,5)P₃ gradient at the leading edge. Furthermore, other oxidant-sensitive, mitogen-activated protein kinase phosphatases such as MKP1 and SHP-2, which are predominantly cytosolic, are not the targets of TRAF4-linked oxidants (18). Thus, TRAF4-p47phox-dependent ROS seem to have specificity for PTP-PEST because of its specific localization at focal contacts and complexes.

In addition to localizing at focal complexes, subunits of NADPH oxidase have also been identified in cell protrusions and membrane ruffles (17, 27). Wu *et al.* (17) had previously reported that through interactions with the cytoskeletal protein WAVE1, p47phox targets to membrane ruffles, where NADPH oxidase stimulates ROS production. This event is required for ruffle formation, tyrosine phosphorylation, and c-Jun N-terminal kinase (JNK) activation (17) that is involved in cell migration (28). Furthermore, upon stimulation with ECs with vascular endothelial growth factor (VEGF), p47phox associates not only with WAVE1 but also with Rac1 and the Rac1 effector PAK1. This leads to the phosphorylation of p47phox by PAK1, which contributes to ROS production and membrane ruffle formation (17) (Fig. 1). Thus, TRAF4 and WAVE1 function as p47phox-binding scaffold proteins to localize a ROS signal at lamellipodial focal complexes and membrane ruffles, respectively, by targeting NADPH oxidase to these specialized membrane compartments and thus stimulating directional cell migration (Fig. 1). Similarly, van Buul *et al.* (27) have shown that Nox2-regulatory subunits, p47phox and p67phox, of NADPH oxidase are found in cell protrusions and membrane ruffles, where they colocalize with Rac1 in primary human ECs.

IQGAP1 is an actin-binding Rac1 effector that links Rac1 to the cytoskeleton and is required for Rac-mediated polarized cell migration (29, 30). Linkage between the microtubule plus-ends and cortical regions is essential for the establishment of cell polarity and directional migration. IQGAP1 captures and stabilizes microtubules by interacting with the microtubule tip-binding protein CLIP-170 near the cell cortical regions (31). Activated Rac1 promotes capture of CLIP-170-capped microtubules in lamellipodia (29). At the leading edge of cells, Rac1 also links the adenomatous polyposis coli (APC) protein to actin filaments through IQGAP1, thereby regulating polarization and directional migration by forming a complex with APC and CLIP-170. Of note, the Nox2 subunit of NADPH oxidase also binds to and colocalizes with IQGAP1 at the leading edge in actively migrating ECs (16). IQGAP1 knockdown using siRNA inhibits Nox2 translocation to the leading edge, ROS production, and cell migration; these findings suggest that, like TRAF4 and WAVE1, IQGAP1 also functions to target Nox2 to specify the site of ROS production during cell migration. IQGAP1 also links Rac1 activation and recruitment of NADPH oxidase to the specific membrane compartments to localize ROS production, thus achieving specificity of redox signaling involved in EC migration (Fig. 1).

Localizing the ROS Signal in Plant Root Cells

Root hair cells of plants become polarized, which is essential for cell growth and development. Foreman *et al.* (32) reported that localized ROS production by NADPH oxidase in the growing tips of cells is required for polarized root hair growth. As in animal cells, ROS production appears to be regulated by Rho GTPases. Carol *et al.* (33) provided the evidence that inactivation of Rho GTPase guanine nucleotide dissociation inhibitor (RhoGDI) encoded by the *supercentipede* (*SCN1*) gene causes more broad distribution of ROS and mislocalization of root hair cells, leading to ectopic hair formation sites. These results suggest that the spatial organization of growth in plant cells requires the local RhoGDI-regulated activation of NADPH oxidase and ROS production. GDI proteins inhibit GTPase activity; thus, it will be intriguing to investigate whether similar negative regulatory mechanisms restrict ROS production and are observed in polarized cell growth of mammalian cells.

Localizing the ROS Signal in Caveolae and Lipid Rafts

Caveolae and lipid rafts are cholesterol- and sphingolipid-rich plasma membrane microdomains where multiple signaling molecules, including G protein-coupled receptors (GPCRs), receptor tyrosine kinases (RTKs), protein kinase C, Src family kinases, and G proteins are localized (34). They serve as platforms for compartmentalization of signaling. Caveolae and its signature protein caveolin-1 (Cav1) are polarized in migrating ECs, indicating their role in cell motility (35). Spatially restricted redistribution of lipid rafts at the leading edge is required for localized activation and coordination of the signaling linked to directed cell migration (36, 37). The polarization of cholesterol-enriched domains changes the plasma membrane microviscosity properties at the front of moving cells, thereby stimulating formation of the actin network required to push the leading-edge membrane forward (38). Furthermore, cell adhesion via integrins regulates targeting of active Rac1 and its coupling of PAK to the plasma membrane by preventing internalization of the lipid rafts (39), which are sites of NADPH oxidase activation (Fig. 1). NADPH oxidases have been identified in caveolae and lipid rafts in various cell systems (40–42), which could be important for the temporal and spatial control of signaling pathways involved in cell migration and the anchorage-dependent cell growth.

In ECs, death receptor activation by TNF- α , Fas ligand, or endostatin stimulates lipid raft clustering and formation of redox signaling platforms in lipid rafts, which contributes to activation of NADPH oxidase and impairment of endothelium-dependent vasorelaxation (41). Using immunofluorescence microscopy and biochemical fractionation, Zhang *et al.* showed that Fas ligand stimulation promotes recruitment of Nox2, p47phox, and Rac1 into lipid rafts, where there is an increase in NADPH oxidase activity and ROS production in ECs (41) (Fig. 2). In vascular smooth muscle cells (VSMCs), Nox1 is found in caveolae and lipid rafts under unstimulated conditions (42). Angiotensin II (Ang II), a GPCR agonist, induces transactivation of epidermal growth factor receptor (EGFR) through redox-sensitive activation of c-Src and c-Abl tyrosine kinases (43, 44). Activation of Src and Abl by Ang II is dependent on Ang II type I receptor (AT₁R) trafficking into Cav1-enriched lipid rafts to which Rac1 is also recruited after Ang II stimulation (45–48). This AT₁R movement is associated with egress of EGFRs from their basal location in Cav1-enriched fractions. Ultimately, transactivated EGFRs migrate with tyrosine-phosphorylated

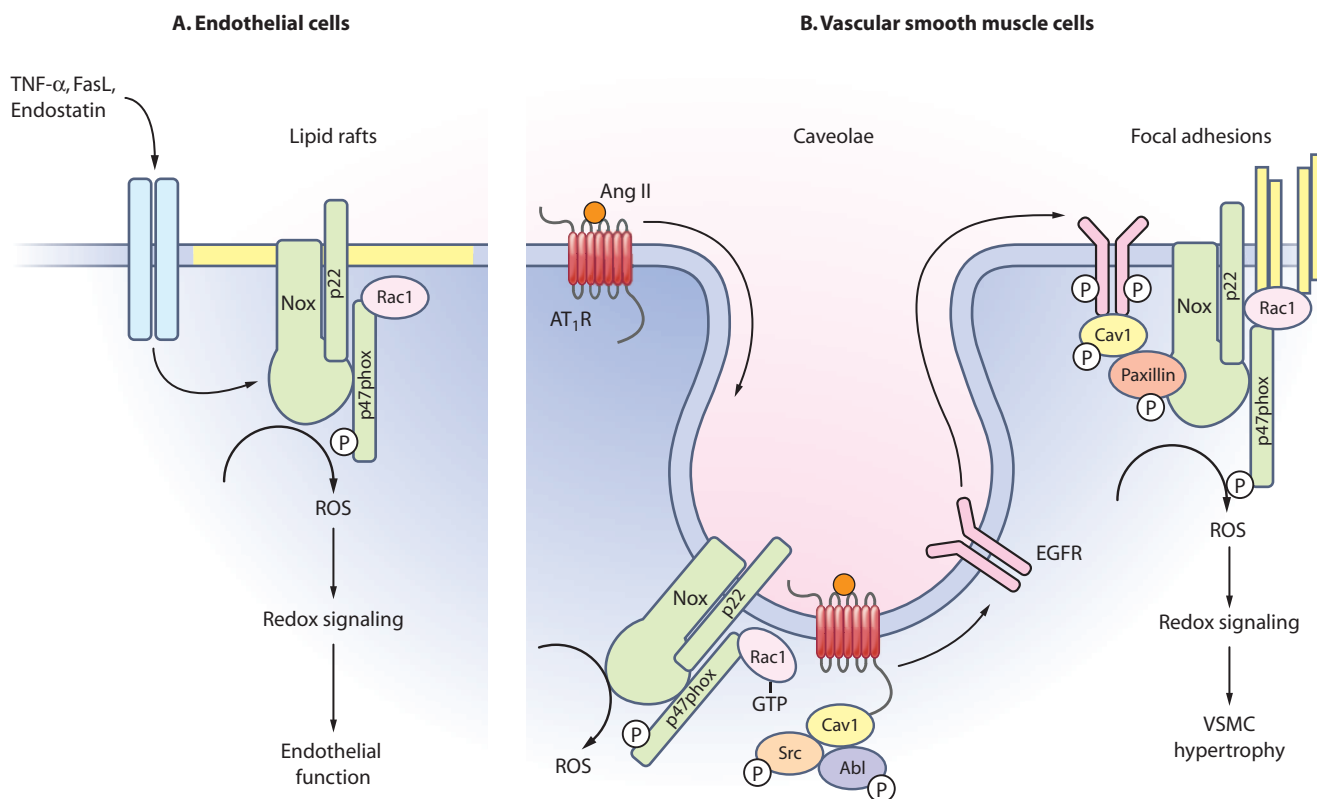


Fig. 2. Localizing the ROS signal in caveolae and lipid rafts. **(A)** In ECs, death receptor activation by TNF- α , Fas ligand, and endostatin stimulates lipid raft clustering and formation of redox signaling platforms in lipid rafts by recruiting Nox2, p47phox, and Rac1, thereby increasing NADPH oxidase activity and ROS production in these specialized membrane microdomains, which contributes to endothelial dysfunction. **(B)** In VSMCs, Nox1 is found in caveolae and/or lipid rafts under unstimulated conditions (42). Ang II stimulation promotes association of caveolin-1, c-Src, and c-Abl with AT₁ receptor and trafficking of receptor complex into caveolae and/or lipid rafts into which Rac1 is also recruited. This AT₁ receptor movement and localized ROS production in specialized microdomains is required for the Ang II–mediated, ROS-dependent transactivation of the EGFR and its egress from caveolae. Tyrosine-phosphorylated EGFR and caveolin-1 subsequently appear at focal adhesions where Nox4 and phospho-paxillin localize, thereby forming redox signaling platforms. These caveolin-1–dependent receptor movements and dynamic assembly of various signal scaffolds with NADPH oxidase are essential for activation of specific redox signaling pathways involved in VSMC hypertrophy.

Cav1 to focal adhesions where Nox4 and phosphorylated paxillin localize, thereby forming redox signaling complexes (44, 46) (Fig. 2). Either inhibition of NADPH oxidase or Cav1 knockdown using siRNA substantially blocks Ang II–induced increase in VSMC hypertrophy measured as an increase in [³H]leucine incorporation (48, 49). These findings indicate that caveolae and lipid rafts play important roles in linking death receptor (Fas) or GPCR (Ang II) signal with NADPH oxidase to promote local production of ROS, thereby forming redox signaling platforms involved in endothelial dysfunction and vascular hypertrophy (Fig. 2). Understanding the functional importance of caveolae and lipid rafts in redox signaling *in vivo* is the subject of future investigation.

Localizing the ROS Signal in the Endosomal and Nuclear Compartments

Several groups have identified subunits of NADPH oxidase on internal membranes. However, a consequence of NADPH oxidase activation in perinuclear regions remains unclear. In unstimulated ECs, Li *et al.* (50) reported that Nox2 and its regulatory proteins exist as a preassembled complex in an intra-

cellular, perinuclear compartment. Moreover, van Buul *et al.* (27) reported that green fluorescent protein (GFP)–Nox4 transfected in primary human ECs is present in an intracellular compartment that colocalizes with a marker for endoplasmic reticulum. In transfected HEK293 cells, Martyn *et al.* (51) showed functional association and colocalization of Nox4 and p22phox at internal membranes. Ambasta *et al.* (52) demonstrated that p22phox–cyan fluorescent protein (CFP) colocalizes with either Nox1–yellow fluorescent protein (YFP) or Nox4–YFP at the endoplasmic reticulum, thereby forming active NADPH oxidase in intracellular compartments.

NADPH oxidase–dependent ROS production in endosomes is important for proinflammatory immune responses. Li *et al.* (53) have demonstrated that interleukin-1 β (IL-1 β) stimulation promotes endocytosis of the IL-1 β receptor (IL-1R1), which is required for Nox2-dependent ROS production at early endosomes and subsequent redox-dependent activation of the transcription factor NF- κ B (Fig. 3). Using a lucigenin assay and electron spin resonance spectroscopy to measure NADPH oxidase activities in isolated vesicular fractions, as well as fluorescence microscopy, they showed that Nox2-dependent O₂⁻ pro-

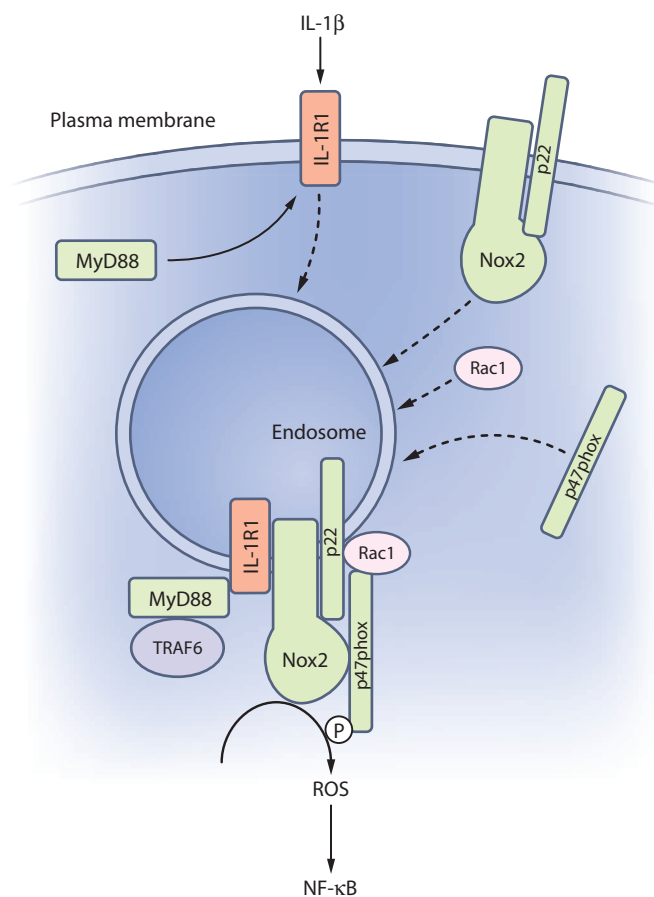


Fig. 3. Localizing the ROS signal in endosomes. Binding of IL-1 β to IL-1R1 on the plasma membrane promotes MyD88 association with IL-1R1, which triggers endocytosis of IL-1R1-MyD88 complex and subsequent recruitment of Rac1 and Nox2 into the endosomal compartment. These events are essential for local NADPH oxidase activation and ROS production by the endosomes. Localized increase in ROS facilitates the redox-dependent association of TRAF6 with the receptor complex on ligand-activated endosomes, which contributes to activation of NF- κ B.

duction was increased in endosomes. IL-1 β binding promotes MyD88 association with IL-1R1, which triggers endocytosis of the IL-1R1/MyD88 complex and subsequent recruitment of Rac1 and Nox2 into the endosomal compartment. Although the mechanism is unknown, Rac1 recruitment into the IL-1R1-containing endosomes is required for translocation of Nox2 from the plasma membrane to this intracellular compartment. Through these processes, endosomes become a source for Nox2-mediated production of H₂O₂, which facilitates the redox-dependent recruitment of TRAF6 to the ligand-activated IL-1R1/MyD88 complex in endosomes to establish the formation of redox-active signaling platforms. This event is required for activation of downstream IKK (inhibitor of NF- κ B kinase) kinases, IKK itself, and ultimately NF- κ B (Fig. 3). Whether similar mechanisms—endocytosis and endosomal ROS generation—are applied to other receptor-mediated redox signaling remains unclear.

Kuroda *et al.* (54) used immunocytochemistry and immunoelectron microscopy to show that the endogenous Nox4 preferentially localizes to the nucleus in human ECs. Nox4 siRNA

abrogates nuclear staining of Nox4, as well as basal- and phorbol ester-stimulated NADPH oxidase activity in the nuclear fraction. Nuclear Nox4-dependent ROS production is involved in oxidative stress-responsive gene expression. Of note, oxidized NF- κ B reduces its DNA binding activity, which is restored by reducing enzymes such as thioredoxin or redox factor 1 (55); these results suggest that the proper redox state in the nuclear compartment is an important determinant for the function of redox-sensitive transcription factors. Thus, it is tempting to speculate that local production of ROS through Nox4 in the nucleus may contribute to regulating redox-dependent gene expression linked to cell growth, differentiation, senescence, and apoptosis. This point should be clarified in future studies.

Conclusion and Future Directions

NADPH oxidase appears to be activated within discrete sub-cellular compartments, including lamellipodial focal complexes and focal adhesions, membrane ruffles, caveolae and lipid rafts, endosomes, and the nucleus, thereby facilitating localized ROS production. These findings provide insights into our understanding of the temporal and spatial organization of agonist-stimulated ROS production and activation of specific redox signaling pathways. It remains to be determined how the sub-cellular localization and targeting of NADPH oxidase are regulated during chemotaxis, proliferation, differentiation, senescence, and apoptosis. Future experiments will reveal the functional importance of localized ROS production, the mechanisms by which targeting of NADPH oxidase is achieved, and also how its activity is controlled to produce ROS in a regulated manner. The future will rely on multiple experimental approaches, including imaging, structural analysis, and molecular approaches, both in vitro and in vivo.

References and Notes

1. M. Ushio-Fukai, R. W. Alexander, Reactive oxygen species as mediators of angiogenesis signaling: Role of NAD(P)H oxidase. *Mol. Cell. Biochem.* **264**, 85–97 (2004).
2. K. K. Griendling, D. Sorescu, M. Ushio-Fukai, NAD(P)H oxidase: Role in cardiovascular biology and disease. *Circ. Res.* **86**, 494–501 (2000).
3. B. M. Babior, NADPH oxidase: An update. *Blood* **93**, 1464–1476 (1999).
4. G. Cheng, Z. Cao, X. Xu, E. G. van Meir, J. D. Lambeth, Homologs of gp91phox: Cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* **269**, 131–140 (2001).
5. C. D. Nobes, A. Hall, Rho, rac and cdc42 GTPases: Regulators of actin structures, cell adhesion and motility. *Biochem. Soc. Trans.* **23**, 456–459 (1995).
6. P. Timpson, G. E. Jones, M. C. Frame, V. G. Brunton, Coordination of cell polarization and migration by the Rho family GTPases requires Src tyrosine kinase activity. *Curr. Biol.* **11**, 1836–1846 (2001).
7. C. M. Laukaitis, D. J. Webb, K. Donais, A. F. Horwitz, Differential dynamics of α 5 integrin, paxillin, and α -actinin during formation and disassembly of adhesions in migrating cells. *J. Cell Biol.* **153**, 1427–1440 (2001).
8. V. S. Kraynov, C. Chamberlain, G. M. Bokoch, M. A. Schwartz, S. Slabaugh, K. M. Hahn, Localized Rac activation dynamics visualized in living cells. *Science* **290**, 333–337 (2000).
9. S. Merlot, R. A. Firtel, Leading the way: Directional sensing through phosphatidylinositol 3-kinase and other signaling pathways. *J. Cell Sci.* **116**, 3471–3478 (2003).
10. M. R. Abid, Z. Kachra, K. C. Spokes, W. C. Aird, NADPH oxidase activity is required for endothelial cell proliferation and migration. *FEBS Lett.* **486**, 252–256 (2000).
11. R. Colavitti, G. Pani, B. Bedogni, R. Anzevino, S. Borrello, J. Waltenberger, T. Galeotti, Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. *J. Biol. Chem.* **277**, 3101–3108 (2002).
12. M. Ushio-Fukai, Y. Tang, T. Fukui, S. Dikalov, Y. Ma, M. Fujimoto, M. T. Quinn, P. J. Pagano, C. Johnson, R. W. Alexander, Novel role of gp91phox-containing NAD(P)H oxidase in vascular endothelial growth factor-induced signaling and angiogenesis. *Circ. Res.* **91**, 1160–1167 (2002).
13. S. Ikeda, M. Ushio-Fukai, L. Zuo, T. Tojo, S. Dikalov, N. A. Patrushev, R. W. Alexander, Novel role of ARF6 in vascular endothelial growth factor-induced signaling and angiogenesis. *Circ. Res.* **96**, 467–475 (2005).

14. H. S. Park, S. H. Lee, D. Park, J. S. Lee, S. H. Ryu, W. J. Lee, S. G. Rhee, Y. S. Bae, Sequential activation of phosphatidylinositol 3-kinase, β Pix, Rac1, and Nox1 in growth factor-induced production of H₂O₂. *Mol. Cell Biol.* **24**, 4384–4394 (2004).
15. L. Moldovan, N. I. Moldovan, R. H. Sohn, S. A. Parikh, P. J. Goldschmidt-Clermont, Redox changes of cultured endothelial cells and actin dynamics. *Circ. Res.* **86**, 549–557 (2000).
16. S. Ikeda, M. Yamaoka-Tojo, L. Hilenski, N. A. Patrushev, G. M. Anwar, M. T. Quinn, M. Ushio-Fukai, IQGAP1 regulates reactive oxygen species-dependent endothelial cell migration through interacting with Nox2. *Arterioscler. Thromb. Vasc. Biol.* **25**, 2295–2300 (2005).
17. R. F. Wu, Y. Gu, Y. C. Xu, F. E. Nwariaku, L. S. Terada, Vascular endothelial growth factor causes translocation of p47phox to membrane ruffles through WAVE1. *J. Biol. Chem.* **278**, 36830–36840 (2003).
18. R. F. Wu, Y. C. Xu, Z. Ma, F. E. Nwariaku, G. A. Sarosi Jr., L. S. Terada, Subcellular targeting of oxidants during endothelial cell migration. *J. Cell Biol.* **171**, 893–904 (2005).
19. S. G. Rhee, Y. S. Bae, S. R. Lee, J. Kwon, Hydrogen peroxide: A key messenger that modulates protein phosphorylation through cysteine oxidation. *Sci. STKE* **2000**, pe1 (2000).
20. P. Chiarugi, P. Cirri, Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem. Sci.* **28**, 509–514 (2003).
21. G. Georgiou, How to flip the (redox) switch. *Cell* **111**, 607–610 (2002).
22. J. Kwon, S. R. Lee, K. S. Yang, Y. Ahn, Y. J. Kim, E. R. Stadtman, S. G. Rhee, Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 16419–16424 (2004).
23. P. Devreotes, C. Janetopoulos, Eukaryotic chemotaxis: Distinctions between directional sensing and polarization. *J. Biol. Chem.* **278**, 20445–20448 (2003).
24. S. Funamoto, R. Meili, S. Lee, L. Parry, R. A. Firtel, Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* **109**, 611–623 (2002).
25. S. K. Sastry, P. D. Lyons, M. D. Schaller, K. Burrridge, PTP-PEST controls motility through regulation of Rac1. *J. Cell Sci.* **115**, 4305–4316 (2002).
26. N. R. Leslie, X. Yang, C. P. Downes, C. J. Weijer, The regulation of cell migration by PTEN. *Biochem. Soc. Trans.* **33**, 1507–1508 (2005).
27. J. D. Van Buul, M. Fernandez-Borja, E. C. Anthony, P. L. Hordijk, Expression and localization of NOX2 and NOX4 in primary human endothelial cells. *Antioxid. Redox Signal.* **7**, 308–317 (2005).
28. C. Huang, Z. Rajfur, C. Borchers, M. D. Schaller, K. Jacobson, JNK phosphorylates paxillin and regulates cell migration. *Nature* **424**, 219–223 (2003).
29. S. C. Mateer, N. Wang, G. S. Bloom, IQGAPs: Integrators of the cytoskeleton, cell adhesion machinery, and signaling networks. *Cell Motil. Cytoskeleton* **55**, 147–155 (2003).
30. M. W. Briggs, D. B. Sacks, IQGAP proteins are integral components of cytoskeletal regulation. *EMBO Rep.* **4**, 571–574 (2003).
31. J. Noritake, T. Watanabe, K. Sato, S. Wang, K. Kaibuchi, IQGAP1: A key regulator of adhesion and migration. *J. Cell Sci.* **118**, 2085–2092 (2005).
32. J. Foreman, V. Demidchik, J. H. Bothwell, P. Mylona, H. Miedema, M. A. Torres, P. Linstead, S. Costa, C. Brownlee, J. D. Jones, J. M. Davies, L. Dolan, S. Costa, Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **422**, 442–446 (2003).
33. R. J. Carol, S. Takeda, P. Linstead, M. C. Durrant, H. Kakesova, P. Derbyshire, S. Drea, V. Zarsky, L. Dolan, A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. *Nature* **438**, 1013–1016 (2005).
34. A. W. Cohen, R. Hnasko, W. Schubert, M. P. Lisanti, Role of caveolae and caveolins in health and disease. *Physiol. Rev.* **84**, 1341–1379 (2004).
35. A. Navarro, B. Anand-Apte, M. O. Parat, A role for caveolae in cell migration. *FASEB J.* **18**, 1801–1811 (2004).
36. S. Manes, R. Ana Lacalle, C. Gomez-Mouton, A. C. Martinez, From rafts to crafts: Membrane asymmetry in moving cells. *Trends Immunol.* **24**, 320–326 (2003).
37. C. Gomez-Mouton, R. A. Lacalle, E. Mira, S. Jimenez-Baranda, D. F. Barber, A. C. Carrera, A. C. Martinez, S. Manes, Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. *J. Cell Biol.* **164**, 759–768 (2004).
38. S. Manes, A. C. Martinez, Cholesterol domains regulate the actin cytoskeleton at the leading edge of moving cells. *Trends Cell Biol.* **14**, 275–278 (2004).
39. M. A. del Pozo, N. B. Alderson, W. B. Kiosses, H. H. Chiang, R. G. Anderson, M. A. Schwartz, Integrins regulate Rac targeting by internalization of membrane domains. *Science* **303**, 839–842 (2004).
40. F. Vilhardt, B. van Deurs, The phagocyte NADPH oxidase depends on cholesterol-enriched membrane microdomains for assembly. *EMBO J.* **23**, 739–748 (2004).
41. A. Y. Zhang, F. Yi, G. Zhang, E. Gulbins, P. L. Li, Lipid raft clustering and redox signaling platform formation in coronary arterial endothelial cells. *Hypertension* **47**, 74–80 (2006).
42. L. L. Hilenski, R. E. Clempus, M. T. Quinn, J. D. Lambeth, K. K. Griendling, Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **24**, 677–683 (2004).
43. M. Ushio-Fukai, K. K. Griendling, P. L. Becker, L. Hilenski, S. Halleran, R. W. Alexander, Epidermal growth factor receptor transactivation by angiotensin II requires reactive oxygen species in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **21**, 489–495 (2001).
44. M. Ushio-Fukai, L. Zuo, S. Ikeda, T. Tojo, N. A. Patrushev, R. W. Alexander, cAbI tyrosine kinase mediates reactive oxygen species- and caveolin-dependent AT1 receptor signaling in vascular smooth muscle: Role in vascular hypertrophy. *Circ. Res.* **97**, 829–836 (2005).
45. N. Ishizaka, K. K. Griendling, B. Lassegue, R. W. Alexander, Angiotensin II type 1 receptor: Relationship with caveolae and caveolin after initial agonist stimulation. *Hypertension* **32**, 459–466 (1998).
46. M. Ushio-Fukai, L. Hilenski, N. Santanam, P. L. Becker, Y. Ma, K. K. Griendling, R. W. Alexander, Cholesterol depletion inhibits epidermal growth factor receptor transactivation by angiotensin II in vascular smooth muscle cells: Role of cholesterol-rich microdomains and focal adhesions in angiotensin II signaling. *J. Biol. Chem.* **276**, 48269–48275 (2001).
47. L. Zuo, M. Ushio-Fukai, L. L. Hilenski, R. W. Alexander, Microtubules regulate angiotensin II type 1 receptor and Rac1 localization in caveolae/lipid rafts: Role in redox signaling. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1223–1228 (2004).
48. L. Zuo, M. Ushio-Fukai, S. Ikeda, L. Hilenski, N. Patrushev, R. W. Alexander, Caveolin-1 is essential for activation of Rac1 and NAD(P)H oxidase after angiotensin II type 1 receptor stimulation in vascular smooth muscle cells: Role in redox signaling and vascular hypertrophy. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1824–1830 (2005).
49. M. Ushio-Fukai, A. M. Zafari, T. Fukui, N. Ishizaka, K. K. Griendling, p22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. *J. Biol. Chem.* **271**, 23317–23321 (1996).
50. J.-M. Li, A. M. Shah, Intracellular localization and preassembly of the NADPH oxidase complex in cultured endothelial cells. *J. Biol. Chem.* **277**, 19952–19960 (2002).
51. K. D. Martyn, L. M. Frederick, K. von Loehneysen, M. C. Dinauer, U. G. Knaus, Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. *Cell. Signal.* **18**, 69–82 (2006).
52. R. K. Ambasta, P. Kumar, K. K. Griendling, H. H. Schmidt, R. Busse, R. P. Brandes, Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. *J. Biol. Chem.* **279**, 45935–45941 (2004).
53. Q. Li, M. M. Harraz, W. Zhou, L. N. Zhang, W. Ding, Y. Zhang, T. Eggleston, C. Yeaman, B. Banfi, J. F. Engelhardt, Nox2 and Rac1 regulate H₂O₂-dependent recruitment of TRAF6 to endosomal interleukin-1 receptor complexes. *Mol. Cell Biol.* **26**, 140–154 (2006).
54. J. Kuroda, K. Nakagawa, T. Yamasaki, K. Nakamura, R. Takeya, F. Kuribayashi, S. Imajoh-Ohmi, K. Igarashi, Y. Shibata, K. Sueishi, H. Sumimoto, The superoxide-producing NAD(P)H oxidase Nox4 in the nucleus of human vascular endothelial cells. *Genes Cells* **10**, 1139–1151 (2005).
55. Y. Kabe, K. Ando, S. Hirao, M. Yoshida, H. Handa, Redox regulation of NF- κ B activation: Distinct redox regulation between the cytoplasm and the nucleus. *Antioxid. Redox Signal.* **7**, 395–403 (2005).
56. Supported by NIH grants HL60728 and HL077524, American Heart Association National Scientist Development Grant 0130175N, and American Heart Association grant-in-aid 0555308B.

Citation: M. Ushio-Fukai, Localizing NADPH oxidase-derived ROS. *Sci. STKE* **2006**, re8 (2006).