Mechanosensitive Regulation of Cortactin by p47\textsuperscript{phox} A New Paradigm in Cytoskeletal Remodeling

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...p47\textsuperscript{phox} is 1 of the 5 subunits that makes up the multisubunit complex of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox2), a major source of cellular reactive oxygen species (ROS). The prototype Nox2 comprises 2 transmembrane subunits (gp91\textsuperscript{phox} [Nox2] and p22\textsuperscript{phox}) and 3 cytosolic subunits (p47\textsuperscript{phox}, p67\textsuperscript{phox}, and p40\textsuperscript{phox}). Nox2 is characterized in phagocytes, where it has a critical antimicrobial function through the production of superoxide anion. Nox2, as well as the other Nox homologues, including Nox1, Nox4, and Nox5, is expressed in many cell types, including those of the cardiovascular system. Although the function of phagocytic Nox2 is clearly defined, the exact pathophysiological significance of cardiovascular Nox2 is still unclear.

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p47\textsuperscript{phox} is a major regulator of Nox2. In the resting state, it localizes in the cytoplasm where its activity is inhibited through an autoinhibitory phosphorylation. However, on cell stimulation, p47\textsuperscript{phox} interacts with cytosolic subunits p67\textsuperscript{phox} and p40\textsuperscript{phox} and the complex translocates to the cell membrane to assemble the active oxidase, which generates superoxide anion. This process is triggered by phosphorylation of p47\textsuperscript{phox}, which is the key for activation of Nox and consequent ROS production. Upregulation of Nox and associated increased ROS generation (oxidative stress) have been implicated in many cardiovascular pathologies, processes that are p47\textsuperscript{phox} dependent because deletion of this subunit reduces oxidative stress and ameliorates pathological cardiac and vascular inflammation and remodeling. On the basis of this paradigm, in this issue of Circulation Research, Patel et al questioned the putative protective effect of p47\textsuperscript{phox} deletion/downregulation in a mouse model of transverse aortic constriction–induced pressure overload and heart failure.

Intriguingly and unexpectedly, p47\textsuperscript{phox} deletion had opposite effects as to what was anticipated, with p47\textsuperscript{phox} knockout mice being susceptible to pressure overload–induced heart failure, adverse myocardial remodeling, and free wall rupture, processes that involve cardiac cytoskeletal disorganization. In a series of elegant studies using p47\textsuperscript{phox}– and Nox2-deficient mice to carefully dissect the impact of p47\textsuperscript{phox} alone versus p47\textsuperscript{phox} as an integral component of Nox2, the authors show unambiguously that the p47\textsuperscript{phox} effect is independent of Nox2, Nox activity, or changes in ROS levels because unlike p47\textsuperscript{phox}–deficient hearts, Nox2-deficient hearts were protected against pressure overload–induced pathological remodeling. Dissecting the ROS-independent mechanisms, whereby p47\textsuperscript{phox} influences cytoskeletal organization, cortactin, a molecular scaffold for actin assembly, was identified as a key player. Such findings challenge the dogma that p47\textsuperscript{phox}, also known as neutrophil cytosolic factor 1, has, as its sole purpose, the organization and activation of Nox1,2 and raise the possibility that p47\textsuperscript{phox} may have pleiotropic functions beyond Nox-derived ROS production.

p47\textsuperscript{phox} and Cortactin: An Enigmatic Partnership

The non–ROS-dependent role of p47\textsuperscript{phox} seems to be linked to organization of the cytoskeleton through processes that involve cortactin (Figure). This relationship between a subunit of the Nox complex and an actin scaffolding protein is intriguing and has several implications. First, p47\textsuperscript{phox} may play a regulatory role in cortactin signaling and actin architecture; second, cortactin-regulated actin may function as a dynamic physical framework that facilitates the trafficking and translocation of the p47\textsuperscript{phox}/p67\textsuperscript{phox}/p40\textsuperscript{phox} complex to the cell membrane to initiate Nox activation; and third, cortactin signaling may influence the phosphorylation pathways involved in activation of p47\textsuperscript{phox}. These paradigms are yet to be proven although previous studies have demonstrated that in vascular cells p47\textsuperscript{phox} interacts physically with the actin cytoskeleton through cortactin to guide translocation of phosphorylated p47\textsuperscript{phox} to the cell membrane.11–14 In this issue, some of these concepts are further developed to show that in the heart, functionally intact p47\textsuperscript{phox} is essential for normal cardiac cytoskeletal dynamics and adaptive remodeling in response to biophysical stress and that in its absence there is a predisposition to cardiac failure through dysregulated cortactin signaling and cytoskeletal disorganization. Hence, at least in the setting of biomechanical stress, p47\textsuperscript{phox} is protective and essential for normal cytoskeletal architecture in the heart, a process that involves N-cadherin, which stabilizes actin and the cytoskeleton.15,16 This phenomenon is dependent on p47\textsuperscript{phox} but not Nox2. These novel findings underscore the importance of the p47\textsuperscript{phox}–cortactin axis in the regulation of actin cytoskeletal networks that are indispensable for normal cell function. To understand how p47\textsuperscript{phox} might act as a cortactin regulator, an appreciation of cortactin biology is necessary.
A Primer in Cortactin Biology

Cortactin, a ubiquitously expressed protein, is a multifunctional regulator of cell migration, invasion, adhesion, and morphogenesis. It possesses an N-terminal acidic domain, a tandem repeat domain (cortactin repeats), a carboxy-terminal proline-rich region containing numerous phosphorylation sites, and an Src homology 3 (SH3) domain. The N-terminus is critical for regulating branched actin assembly through interactions with the branched actin-nucleating actin-related protein 2/3 and filamentous actin at the acidic and repeat domains, respectively. Virtually, all the cellular functions of cortactin require association with actin-related protein 2/3 complex and the actin cytoskeleton.

Although the N-terminus domain is directly engaged in actin assembly, the C-terminus is particularly important in cytoskeletal organization because it acts as a scaffolding protein for binding many interacting proteins through the proline-rich region and SH3 domains. In fact, ≥15 cortactin-binding proteins that target the SH3 domain have been identified, including GTPase regulators (eg, BPGAP1), GTPase (dynamin2), and adaptor/scaffolding proteins (Wiskott-Aldrich syndrome protein, missing-in-metastasis, nonmuscle myosin light chain kinase, and neural Wiskott-Aldrich syndrome protein), indicating the diverse roles of cortactin-associated actin networking. Cortactin is also regulated by phosphorylation through receptor tyrosine kinases, nonreceptor tyrosine kinases, and serine/threonine kinases. It in this context that p47phox may be added to the list of cortactin/actin regulators because p47phox itself contains SH3 and proline-rich domains through which it could interact with cortactin, and it is also regulated through phosphorylation/dephosphorylation processes.

**p47phox as a Putative Cortactin Regulator**

p47phox has several functional domains: a phox homology domain, 2 SH3 domains, an autoinhibitory region, a proline-rich domain, and several phosphorylated sites. In resting conditions, the 2 SH3 domains interact intramolecularly with the C-terminal domain of nonphosphorylated protein to maintain p47phox in an autoinhibited state. On cell stimulation, p47phox is sequentially phosphorylated on a number of serines located between S303 and S379. Phosphorylation of S379 is a key for oxidase activation. Importantly, p47phox possesses an actin-binding site, which is 1 of the strongest indicators that this protein interacts with actin/cytoskeletal proteins. Numerous protein kinases have been implicated in the phosphorylation of p47phox, including protein kinase C, Akt, extracellular signal-regulated kinase 1/2, Src, and p21-activated kinase. Some kinases, such as protein kinase A and casein kinase II, influence p47phox through a negative inhibitory effect. Of note, many of the kinases that stimulate phosphorylation of p47phox also induce phosphorylation of cortactin.

In resting cells, all 100% of p47phox is located within the cytosol. During activation, only 10% to 20% of p47phox migrates to the plasma membrane to associate with Nox/p22phox. This means that 80% to 90% of phosphorylated p47phox remains in the cytosol, where it may have Nox/ROS-independent functions through its binding to the cytoskeleton, an effect that may occur through interactions with cortactin at the SH3 or proline-rich region domains or binding of the phox homology domain to moesin, as previously demonstrated. Taken together, there are many mechanisms, whereby p47phox could interact with cortactin and the cytoskeleton; however, these still need to be definitively demonstrated and the biological significance of such interactions needs more investigation.

**p47phox and the Cytoskeleton: A New Paradigm**

Although the findings of Patel et al are certainly exciting because they question current doctrine related to p47phox biology, there are a number of aspects that merit further consideration. First, it is unknown whether the described deleterious cardiac effects of p47phox downregulation...
relate specifically to biomechanical stress. Second, the mechanosensor/transducer linking physical stress to the p47<sup>phox</sup>/cortactin/actin network is unknown. Third, the potential influence of the p47<sup>phox</sup> isoform, Nox organizer 1, in the setting of p47<sup>phox</sup> deletion is not considered, and finally, it is unclear whether the p47<sup>phox</sup>-related processes are cardiac-specific. Furthermore, it still remains unclear why in the model of transverse aortic constriction–induced pressure overload, p47<sup>phox</sup> deletion aggravates the pathological outcomes, whereas in almost all other experimental models of cardiovascular disease, p47<sup>phox</sup> deletion/downregulation is associated with cardiovascular protection.4,7,29 Perhaps, this relates to differential activation of the non–ROS-dependent versus the ROS-dependent pathways of p47<sup>phox</sup).

Despite the limitations, the study by Patel et al is important because it advances the field in many ways, by showing that p47<sup>phox</sup> has Nox/ROS-independent functions. These findings are provocative because they question the exclusive role of the Nox subunits and suggest that these subunits may have dual or multiple functions. Indeed even with regard to ROS generation, p47<sup>phox</sup> has a complex dual role because it inhibits basal Nox activity but is critical for angiotensin II–induced vascular dysfunction via activation of Nox.8,30 The time is now ripe to address the multifunctionality of Nox subunits, especially with the availability of innovative transgenic mouse models and molecular tools to comprehensively interrogate the Nox system. Furthermore, from the clinical point of view, it would be particularly enlightening to know whether patients with chronic granulomatous disease caused by p47<sup>phox</sup> mutations are more susceptible to cardiac pathologies than those with Nox2 (gp91<sup>phox</sup>) mutations. Such studies would provide a perfect human correlate to the experimental paradigm under discussion. This is an exciting time in Nox science and perhaps it won’t be too long before we learn that Nox indeed has functions beyond ROS formation. Such novel concepts will transform the landscape in Nox/redox biology and may explain why, despite Nox hyperactivation and increased oxidative stress in many pathologies, antioxidants therapies have failed. Perhaps, there are Nox-related molecules beyond ROS, such as p47<sup>phox</sup>/cortactin, that may be more important targets. This interesting thesis demands further investigation.

Sources of Funding
Quoted studies by the authors were funded by grants from the Canadian Institute of Health Research and the Heart and Stroke Foundation of Canada.

Disclosures
None.

References


**Key Words:** actin ■ cardiac failure ■ mechanotransduction ■ NADPH oxidase ■ reactive oxygen species
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*Circ Res.* 2013;112:1522-1525
doi: 10.1161/CIRCRESAHA.113.301495

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/112/12/1522

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