Mechanosensitive Regulation of Cortactin by p47phox
A New Paradigm in Cytoskeletal Remodeling

Augusto C. Montezano, Rhian M. Touyz

p47phox 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, p47phox interacts with cytosolic subunits p67phox and p40phox and the complex translocates to the cell membrane to assemble the active oxidase, which generates superoxide anion. This process is triggered by phosphorylation of p47phox, 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 p47phox 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 p47phox deletion/downregulation in a mouse model of transverse aortic constriction–induced pressure overload and heart failure.

Intriguingly and unexpectedly, p47phox deletion had opposite effects as to what was anticipated, with p47phox 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 p47phox- and Nox2-deficient mice to carefully dissect the impact of p47phox alone versus p47phox as an integral component of Nox2, the authors show unambiguously that the p47phox effect is independent of Nox2, Nox activity, or changes in ROS levels because unlike p47phox-deficient hearts, Nox2-deficient hearts were protected against pressure overload–induced pathological remodeling. Dissecting the ROS-independent mechanisms, whereby p47phox influences cytoskeletal organization, cortactin, a molecular scaffold for actin assembly, was identified as a key player. Such findings challenge the dogma that p47phox, also known as neutrophil cytosolic factor 1, has, as its sole purpose, the organization and activation of Nox1,2 and raise the possibility that p47phox may have pleiotropic functions beyond Nox-derived ROS production.

p47phox and Cortactin: An Enigmatic Partnership

The non–ROS-dependent role of p47phox 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, p47phox 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 p47phox/p67phox/p40phox complex to the cell membrane to initiate Nox activation; and third, cortactin signaling may influence the phosphorylation pathways involved in activation of p47phox. These paradigms are yet to be proven although previous studies have demonstrated that in vascular cells p47phox interacts physically with the actin cytoskeleton through cortactin to guide translocation of phosphorylated p47phox to the cell membrane. In this issue, some of these concepts are further developed to show that in the heart, functionally intact p47phox 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, p47phox is protective and essential for normal cytoskeletal architecture in the heart, a process that involves N-cadherin, which stabilizes actin and the cytoskeleton. This phenomenon is dependent on p47phox but not Nox2. These novel findings underscore the importance of the p47phox–cortactin axis in the regulation of actin cytoskeletal networks that are indispensable for normal cell function. To understand how p47phox 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.\(^9,10\) 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.\(^1,10\) Virtually, all the cellular functions of cortactin require association with actin-related protein 2/3 complex and the actin cytoskeleton.\(^9,10,18\)

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.\(^9,10\) Cortactin is also regulated by phosphorylation through receptor tyrosine kinases, nonreceptor tyrosine kinases, and serine/threonine kinases.\(^9,10,18\) It is in this context that p47phox may be added to the list of cortactin/actin regulators because p47phox itself contains SH3 and proline-rich region 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.\(^1,2\) 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.\(^20\) Importantly, p47phox possesses an actin-binding site, which is 1 of the strongest indicators that this protein interacts with actin/cytoskeletal proteins.\(^21,22\) 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.\(^23–25\) 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, ≈100% of p47phox is located within the cytosol.\(^1\) 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.\(^1\) 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\(^6\) 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 p47phox/cortactin/actin network is unknown. Third, the potential influence of the p47phox isoform, Nox organizer 1, in the setting of p47phox deletion is not considered, and finally, it is unclear whether the p47phox-related processes are cardiac-specific. Furthermore, it still remains unclear why in the model of transverse aortic constriction–induced pressure overload, p47phox deletion aggravates the pathological outcomes, whereas in almost all other experimental models of cardiovascular disease, p47phox deletion/downregulation is associated with cardiovascular protection.4,7,20 Perhaps, this relates to differential activation of the non–ROS-dependent versus the ROS-dependent pathways of p47phox.

Despite the limitations, the study by Patel et al is important because it advances the field in many ways, by showing that p47phox 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, p47phox has a complex dual role because it inhibits basal Nox activity but is critical for angiotensin II–induced vascular dysfunction via activation of Nox.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 p47phox mutations are more susceptible to cardiac pathology.1 Moreover, from the clinical point of view, it would be particularly enlightening to know whether patients with chronic granulomatous disease caused by p47phox mutations are more susceptible to cardiovascular disease, p47phox deletion/downregulation is associated with cardiovascular protection.4-7,20 Perhaps, this relates to differential activation of the non–ROS-dependent versus the ROS-dependent pathways of p47phox.

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References


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