ox4, one of the 7 isoforms of the NADPH (nicotinamide adenine dinucleotide phosphate) oxidase family that generate reactive oxygen species, was first identified in the early 2000s in kidneys.\(^1\) Nox4 was originally described as an oxygen sensor and regulator of kidney cell growth and because of its renal abundance was termed “renox.”\(^1\) It soon became evident that Nox4 has a ubiquitous distribution and that it is expressed in many organ systems, such as the vasculature, and in many cell types including endothelial and vascular smooth muscle cells and in adventitial fibroblasts and adipocytes.\(^2\)

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Nox4 is a unique Nox isoform. It localizes in the plasma membrane as well as intracellularly (focal adhesions, nucleus, mitochondria, endoplasmic reticulum), is constitutively active, is regulated at the gene level, associates with novel regulators such as Poldip2, and is influenced by vasoactive agonists (angiotensin II), physical factors (shear stress and flow), and others such as hypoxia, and microRNAs (miRNA25).\(^2–4\) Nox4 associates with p22phox for its activation, does not require interaction with classic NADPH oxidase cytosolic subunits (p47phox, p67phox, p40phox), and, unlike other Noxs, generates H\(_2\)O\(_2\) in preference to O\(_2\)\(^{-}\). Despite the fact that there has been enormous advancement in the understanding of Nox4 biochemistry and the mechanisms of Nox4 regulation, the functional significance and biological role of Nox4 remain elusive. Most studies investigating the (patho)physiology of Nox4 in the vascular system have become available, and the data deriving from such models have yielded surprising results because, unexpectedly, and as demonstrated in the study by Schroder et al in the current issue,\(^14\) it seems that Nox4 may in fact be vascular-protective rather than vascular-damaging.

Genetically modified mouse strains to investigate the roles of Nox isoforms in experimental models of vascular disease have been limited to Nox1 and Nox2.\(^13\) It is only very recently that Nox4 transgenic and knockout mouse models have been available, and the data deriving from such models have yielded surprising results because, unexpectedly, and as demonstrated in the study by Schroder et al in the current issue,\(^14\) it seems that Nox4 may in fact be vascular-protective rather than vascular-damaging.

The first study investigating direct in vivo effects of Nox4 on vascular function was reported in 2011 by Ray et al,\(^15\) in which gene-modified mice with endothelium-targeted Nox4 overexpression exhibited enhanced vasodilation and reduced basal blood pressure. Key mechanisms underlying the vasodilator effect of Nox4 were attributed to vasodilator effects of increased H\(_2\)O\(_2\) production and reduced NO inactivation. In support of these findings, Schroder et al\(^14\) show that in global Nox4 knockout mice, ischemia-induced angiogenesis is attenuated and that in tamoxifen-inducible Nox4 knockout mice, angiotensin II–mediated aortic inflammation, vascular remodeling, and endothelial dysfunction are exaggerated, effects associated with decreased eNOS expression, reduced NO generation, and blunted heme oxygenase-1 expression. In line with these data, others showed that in transgenic mice with endothelial-specific Nox4 overexpression (VECad-
Nox4 mice), recovery from hind limb ischemia was enhanced with increased aortic capillary sprouting.\textsuperscript{16} Collectively, these data suggest that endothelial Nox4 promotes angiogenesis and recovery from hypoxia. Molecular mechanisms implicated in Nox4 vasoprotection include activation of eNOS and increased production of the vasodilator NO, generation of H\textsubscript{2}O\textsubscript{2}, a putative endothelium-derived relaxing factor, and increased expression/activation of antioxidant systems such as Nrf-2, a master regulator of antioxidant genes. Hence, based on these recent studies using Nox4 transgenic and knockout mouse models, a new paradigm is emerging that Nox4 may be vasculoprotective and that upregulation of this Nox isoform may have potential therapeutic benefit in preventing vascular disease. However, data from Nox4\textsuperscript{-/-} mice are not consistent. In models of transient or permanent stroke, Nox4-deficient mice and mice treated with the Nox4 inhibitor VAS2870 were protected from oxidative stress, blood-brain barrier leakage, and neuronal apoptosis.\textsuperscript{17} These data suggest that Nox4 is a major source of reactive oxygen species in ischemic stroke and that inhibition of Nox4 may be a strategy for stroke therapy.\textsuperscript{17}

To reconcile the growing confusion in the field, a number of considerations warrant further discussion. First, in the study of Schroder et al.,\textsuperscript{14} vascular functional studies were performed in large conduit vessels (aorta) and in lung endothelial cells from Nox4-deficient mice, whereas many previous studies explored small arteries and cultured vascular smooth muscle cells.\textsuperscript{3,5,9,10,11,17,18} Second, in Nox4 knockout mice, the gene deletion is global, and, accordingly, it is not entirely correct to implicate a vascular-specific pathology in a model in which there is a ubiquitous knockout. Third, the fact that it is only in challenged conditions that Nox4 knockout mice exhibit a vascular phenotype indicates a nonessential role of Nox4 in physiological conditions. Fourth, the experimental paradigms focused on ischemic injury and angiotensin II–induced oxidative stress. It may be possible that under diverse conditions of stress and challenge, regulation of Nox4 may be very different, for example, angiotensin II, ET-1, TGF\textbeta, and stretch differentially regulate Nox4 in vitro and in whole-animal studies.\textsuperscript{18,19} Fifth, recent evidence indicates that Nox4 regulates its own expression. Overexpression of Nox4 in adventitial myofibroblasts inhibits endogenous Nox4-mediated cell signaling, suggesting that Nox4 causes feedback inhibition of its own expression.\textsuperscript{20} Such interactions may be important in mice in which Nox4 is overexpressed, where Nox4 autoregulation of its own expression may affect vascular function and phenotype. Finally, the experimental conditions in which Nox4 is genetically manipulated must be considered because different approaches may result in opposing outcomes. In fact, in some conditions, even the same approach yields divergent results. Mice in which Nox4 was targeted in a cardiac-specific manner demonstrated that Nox4 is both protective\textsuperscript{21} and injurious\textsuperscript{22} in models of cardiac overload.

The findings of Schroder et al\textsuperscript{14} and others\textsuperscript{15–17} certainly challenge the dogma and are most thought-provoking. They also highlight the need to understand why conclusions from in vitro studies are opposite to those from in vivo studies and why data from Nox4\textsuperscript{-/-} mice yield contradictory results.\textsuperscript{14–21} At the molecular level, the divergent effects may relate, at least in part, to the relative proportion of H\textsubscript{2}O\textsubscript{2} versus O\textsubscript{2}\textsuperscript{-} produced (Figure). Further studies to elucidate the exact (patho)physiological role of Nox4 are warranted, and, now that Nox4 knockout mice and new tools to interrogate Nox4 are available for investigation, we should have a clearer understanding of what Nox4 does in physiological and pathological conditions. If future studies provide unambiguous evidence that Nox4 is indeed vascular-protective, the therapeutic potential of Nox4 inhibitors, which are currently being developed for clinical use,\textsuperscript{23} comes under question because such strategies may aggravate rather than ameliorate vascular damage. There is thus an urgent need to unravel the Nox4 paradox.

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