The Reactive Adventitia
Fibroblast Oxidase in Vascular Function
Federico E. Rey, Patrick J. Pagano

Abstract—The vascular adventitia is activated in a variety of cardiovascular disease states and has recently been shown to be a barrier to nitric oxide bioactivity. Vascular fibroblasts produce substantial amounts of NAD(P)H oxidase–derived reactive oxygen species (ROS) that appear to be involved in fibroblast proliferation, connective tissue deposition, and perhaps vascular tone. However, the physiological and pathophysiological roles of the adventitia have not been extensively studied, possibly because of its location in large blood vessels remote from the vascular endothelium. In recent years, substantial information has been gathered on pathways leading to oxidase activation in smooth muscle cells and fibroblasts and the downstream signaling pathways leading to hypertrophy and proliferation. A clearer understanding of the molecular mechanisms involved will likely lead to therapeutic strategies aimed at preventing vascular dysfunction in diseases such as atherosclerosis, in which these pathways are activated. (Arterioscler Thromb Vasc Biol. 2002;22:1962-1971.)

Key Words: NAD(P)H oxidase ■ NADPH oxidoreductase ■ fibroblast ■ vascular smooth muscle ■ adventitia ■ remodeling

The contribution of the adventitia to vascular function has largely been ignored, except for an occasional mention that it provides support for the blood vessel (extracellular matrix) and a scaffold for sympathetic nerve endings and the vasa vasorum. Much attention has been given to the vascular endothelium in the past 20 years since the discovery of endothelium-derived relaxing factor (EDRF), but before that, it was held in the same disregard as the adventitia, considered merely a physical barrier separating tissues. The adventitia is defined as “the outermost connective tissue of any organ, vessel, or other structure not covered by a serosa; instead, the covering is properly derived from without . . . and does not form an integral part of such organ or structure”1 and is derived from the Latin adventicius, or “coming from abroad, foreign.” Thus, given its “outsider” status, it is no surprise that the adventitia has not been given substantial consideration. In recent years, several important studies have lent credence to the concept that functional changes in the adventitia lead to vascular pathology. The rapid growth of the field of reactive oxygen species (ROS) and the realization that these species can act as tissue-signaling agents have brought new significance to the role of the adventitia in vascular function. Hopefully, this review may dispel some of the obscurity clouding this important tissue.

Anatomy of a Blood Vessel
The most commonly studied arterial segments are (1) the tunica intima, consisting of a longitudinal endothelial lining and, in some vessels, containing delicate connective tissue with occasional smooth muscle cells (SMCs) and (2) the tunica media, made up of circumferentially arranged SMCs and, depending on its size, a well-developed elastic net. The third segment, called the tunica adventitia, is composed primarily of fibroblasts, collagen, and elastin fibers oriented longitudinally. A variety of other cells, including mast cells, macrophages, and ganglionic cells, are also present. The relative size of the 3 segments varies depending on the size of the artery. Interestingly, medial thickness ranges from 3 or 4 smooth muscle layers in small arteries to 40 in large arteries.2 The boundary between the intima and media is demarcated by the internal elastic lamina, and that between the media and adventitia by the external elastic lamina. The adventitia gradually merges with loose connective tissue around the vessel, often surrounded by a layer of adipose tissue.2,3

The Adventitia as a “Launching Pad” for Ameliorative Vasoactive Agents
Because of the apparent ease of surgical access and the interest in averting endothelial damage, recent studies have used gene transfer to the adventitia as a means of correcting vascular dysfunction. After successful transfection of cerebral vessel adventitial cells was demonstrated by intracerebroventricular injections of adenovirus, a series of landmark studies has shown that the adventitia is a useful platform for expression of tissue-permeant hormones such as nitric oxide...
Activation of the Adventitia in Vascular Disease

Hypertension, atherosclerosis, and vascular injury all activate perivascular cells and increase macrophage levels in the perivascular space. As far back as 1915, Allbutt reported finding inflammatory cells in the adventitia of atherosclerotic arteries. In 1962, Schwartz and Mitchell demonstrated a positive relationship between the degree of adventitial inflammation and severity of atherosclerosis. Combined with leukocyte infiltration, characteristic fibroblast proliferation will likely increase perivascular production of O₂⁻ and impair endothelium-dependent relaxation (EDR). Generation of angiotensin II (AngII) and cytokines by perivascular adipose tissue and the interstitium, as well as macrophages and mast cells present in the adventitia, could also potentiate endogenous O₂⁻ production and lead to the production of other ROS, including hydrogen peroxide (H₂O₂), which is cell-permeant and likely to affect vascular smooth muscle responsiveness. In fact, mast cells do contain a leukocyte-like NADPH oxidase. Adventitial NAD(P)H oxidase activity may affect nitricergic neurotransmission, since adventitial fibroblasts are juxtaposed to adventitial nerve endings that produce NO. Therefore, in vivo adventitial O₂⁻ may play an even more extensive role in the control of vascular tone than the one observed in vitro.

Interactions of Adventitial ROS With EDRF

Almost immediately after the discovery of EDRF, it was surmised that O₂⁻ was involved in its destruction. It was later discovered that EDRF is NO, a free radical that is essentially inactivated by reaction with O₂⁻. Clearly, O₂⁻ can interfere with NO-dependent vasodilatation and participate in endothelium-dependent constriction. An inducible phagocyte-like NAD(P)H oxidase has been reported in the endothelium and smooth muscle and has been cited in the regulation of NO bioactivity. Several key studies described a functional oxidase in these cells and implicated its contribution to impaired endothelial function. Because these O₂⁻ sources are near the site of endothelial NO synthase (eNOS)–derived NO, it is broadly accepted that they interfere with the actions of endothelium-derived NO (EDNO). Our early studies revealed that a major site of vascular O₂⁻ production was derived from adventitial NAD(P)H oxidase. Although it was not immediately clear how the adventitia might be involved in the elimination of exogenously applied NO, it was implicated just the same by virtue of its action as a physical barrier. Wang et al showed that this destruction was caused by adventitial O₂⁻ and went on to address the significance of the adventitia for bioactivity of EDNO by demonstrating that it interacts with adventitial O₂⁻ to enhance passive aortic tone. Moreover, recent reports have shown hormonal induction of medial SMC NO, further supporting the interaction between vascular NO and adventitial O₂⁻. It was already well known that superoxide dismutase (SOD) can improve relaxation of blood vessels and it was later found that xanthine oxidase plays a role in the development of high blood pressure in spontaneously hypertensive rats. Our group and others have inferred that O₂⁻ derived from NAD(P)H oxidase inhibits NO-dependent relaxation, and vascular O₂⁻ from this oxidase is now implicated in AngII-induced blood pressure elevation. Although it is intuitive that endothelial and medial sources of O₂⁻ would be impediments to NO, it has not been so clear whether adventitial O₂⁻ can substantially inactivate EDNO. Yet a large source of O₂⁻ in the adventitia is relevant to EDNO bioactivity. Beckman and Koppenol describe O₂⁻ as a major scavenger of NO that can act as a sink and lower its bioactive concentrations over its diffusion radius of 150 to 300 μm. This phenomenon is related to the ability of NO to diffuse in a Brownian pattern faster than it reacts with most biological substances, including the heme in guanylate cyclase (diffusion rate of 3300 μm/s under physiological conditions). Relevant to this point, the medial thickness of an adult rat common carotid artery is ~60 μm. Thus, NO is expected to travel to the adventitia and be inactivated by any major source of O₂⁻ within NO’s diffusion radius before it can maximally activate guanylate cyclase in the media. Our most recent experiments suggest that adventitial O₂⁻ interferes with EDNO-induced relaxation of the normal mouse aorta. By compartmentalizing the aortic endothelium from the adventitia by isolated perfusion and suffusion, we were able to demonstrate that AngII-induced impairment of EDR was significantly improved by localized delivery of SOD to the adventitia. Based on the premise that O₂⁻ has a diffusion radius of a few microns, these studies suggest that adventitial O₂⁻ (1) acts as a sink for vascular NO (Figure 1A) and/or (2) constricts the outer vascular medial layers or stimulates vasoconstrictor release from the adventitia and (3) stimulates the release of a paracrine mediator of smooth muscle relaxation impairment (Figure 1B). For instance, ROS activates cyclooxygenase and enhance the vasoconstrictor action of prostaglandin H₂. They also stimulate vascular smooth muscle cells (VSMCs) to release heat-shock protein 90α and cyclophilins, which may activate extracellular signal-regulated kinases (ERK1/2) in an autocrine fashion and mediate SMC contraction. Possible paracrine mediators of this impairment include cytokine and growth factor release derived from fibroblasts and mast cells. In fact, the interaction of mast cells with fibroblasts in cardiovascular disease could become an important area of study, because mast cell mediators stimulate fibroblast growth and collagen synthesis. Figure 1 illustrates the possible mechanisms by which ROS may directly or indirectly affect the constrictor tone of vascular smooth muscle. Moreover, evidence demonstrating that leukocytes accumulate in the adventitia in the early stages of cardiovascular disease suggests a role for leukocytes in vascular dysfunction. Interactions of invading macrophages and fibroblast oxidase is also an area of active interest in our laboratory.
Role of the Vascular Adventitia in Vascular Remodeling

Proliferation of fibroblasts modulated by O$_2^-$ and a change in the balance of matrix development by fibroblasts could ultimately lead to changes in SMC and endothelial cell growth and vessel dynamics. Indeed, adventitial fibroblasts have been described as "most reactive in the vascular wall" and appear to initiate vascular remodeling in response to injury.

In hypertension, medial hypertrophy of large arteries is a normal response, yet the mechanism mediating this hypertrophy is still unclear. Numerous reports have demonstrated that AngII can induce medial thickening and increase cross-sectional area independently of blood pressure elevation. Other reports argue that pressure mediates much of this effect. In SMC cultures, AngII has clearly been shown to induce hypertrophy, which is mediated by activation of NAD(P)H oxidase–derived H$_2$O$_2$; this in turn activates proto-oncogenes, ERK1/2, and transcription factors, leading to the growth response. However, involvement of neighboring cells in medial hypertrophy has not been reported to our knowledge. Inasmuch as studies support activation of adventitial NAD(P)H oxidase by AngII and that Fukai et al have reported that AngII increases SOD, it is tempting to speculate that H$_2$O$_2$ resulting from adventitial NAD(P)H oxidase activation affects medial SMCs in a variety of ways, including stimulation of hypertrophy and decreased sensitivity of guanylate cyclase. In fact, our data suggest that adventitial NAD(P)H oxidase produces O$_2^-$ outside the cell, which could plausibly be converted by extracellular SOD to H$_2$O$_2$, a more stable and cell-permeant stimulator of medial smooth muscle hypertrophy. A recent report by Liao et al showed that oxidative stress in SMCs causes the release of
heat-shock protein 90α, which can activate ERK1/2 in other cells. This important study strongly supports a paracrine effect of oxidative stress in the vasculature, and if this same mechanism exists in adventitial fibroblasts, it may help explain how fibroblast O$_2^-$ effects medial hypertrophy. More recently, Wang et al$^{64}$ showed that AngII stimulates NADPH oxidase–derived ROS in the adventitia and intima concomitant with medial hypertrophy. This stimulation was significantly reduced in mice without gp91$^{\text{phox}}$-containing NAD(P)H oxidase, suggesting a paracrine interaction between the media and adjacent vascular layers. Finally, stimulation of the adventitia has been clearly associated with transmigration of adventitial fibroblasts into myofibroblasts,$^{54}$ cells that are known to be constrictive and produce large amounts of extracellular matrix.$^{65,66}$ Both of these characteristics of the myofibroblast are expected to lead to vascular remodeling.

Vascular Adventitia in the Injury Response

VSMC migration and proliferation have been implicated in narrowing of the arterial lumen in response to injury and atherosclerosis. Although the mechanisms involved are not fully understood, AngII, growth factors, and proto-oncogenes have been suggested. Angiotensin-converting enzyme inhibitors (ACEIs) can prevent neointima formation in response to balloon injury.$^{67,68}$ and AngII receptor antagonists may also inhibit neointima formation.$^{68}$ These data suggest that AngII is involved in the process leading to neointimal thickening by way of increased SMC proliferation.$^{69}$ There is little doubt that SMCs proliferate and migrate to the neointima. However, because AngII induces hypertrophy but not proliferation of VSMCs in culture,$^{31,70}$ it is not so clear whether AngII induces migration of SMCs to the neointima. Recently, the adventitia has been shown to play an important role in the remodeling response to injury under normotensive conditions. It has been postulated that fibroblast proliferation precedes modulation to myofibroblasts, which then migrate to the neointima.$^{54}$ Inasmuch as fibroblast proliferation is O$_2^-$ dependent,$^{52}$ therapies aimed at targeting O$_2^-$ production in adventitial fibroblasts may prove useful in treating both the vascular injury response and atherosclerosis. Although the inherent differences among various vascular beds and models of vascular injury are obvious, there is increasing evidence that the adventitia plays a significant role. For example, Patel et al$^{71}$ have shown that SMCs are more or less differentiated, depending on the vascular bed. This may explain why in coronary arteries, where they are more differentiated, SMCs do not proliferate and migrate. Also, in a porcine model of coronary artery balloon injury used by Shi et al$^{72}$ dissection was produced by varying degrees of medial injury, and intima-bound myofibroblasts were found to migrate along medial fissures. However, dissection of the media is not necessary, because direct adventitial injury can cause neointimal lesions even in the absence of endothelial denudation.$^{71,73}$ Furthermore, Shi et al$^{74}$ recently showed that in carotid artery-vein grafts, neointimal proliferation is preceded by activation and proliferation of adventitial fibroblasts, modulation to myofibroblasts, and migration to the neointima. Indeed, Hollifield et al$^{75}$ have described carotid adventitial fibroblasts as far more likely to proliferate than carotid SMCs, and Li et al$^{76}$ showed that in the rat carotid injury model, exogenously modified and seeded carotid adventitial fibroblasts migrate in response to a factor released by SMCs. However, there remains significant controversy over the relative contribution of medial SMCs and adventitial fibroblasts in neointimal growth. The increased presence of p22$^{\text{phox}}$ in dedifferentiated smooth muscle in the vein graft neointima,$^{77}$ as well as the upregulation of vascular smooth muscle Nox1 during restenosis,$^{78}$ clearly support a role for medial SMCs in this process, as suggested by early studies.$^{79,80}$ Moreover, in contrast to the studies by Li et al,$^{76}$ a recent report by de Leon et al$^{81}$ suggests that resident fibroblasts do not migrate from the adventitia in the rat carotid artery injury model. The differences in the contribution of adventitial nonmuscle cells in various vascular beds are likely to be traced to the arteries being studied and the degree of injury sustained. With regard to the former, the developmental origin of SMCs and fibroblasts is likely to ascribe a different proliferative and migratory phenotype to SMCs, depending on their origin.$^{71}$ Regardless of the origin of the neointimal cells, there appears to be little doubt that the adventitia plays an important role (whether direct or indirect) in neointima development in these models. Perhaps indirect influences include adventitial release of transforming growth factor-β in response to an increase in ROS levels, which causes cell proliferation.$^{82}$ Our recent studies targeting NAD(P)H oxidase in adventitial cells with adenoviral vectors expressing oxidase inhibitor have revealed substantial reductions in O$_2^-$ and neointimal growth, suggesting interaction between adventitial oxidase and vascular cell proliferation and migration.$^{83}$ Cross-talk between the vascular segments is currently a focus of intense interest in our laboratory.

Role of the Adventitia in Atherosclerosis

A variety of studies have suggested that the adventitia is activated during the development of atherosclerosis.$^{13,84,85}$ AngII is known to induce ROS formation in vitro and in vivo, and oxidative stress plays a role in hypercholesterolemia, atherosclerosis, and vascular injury.$^{86,87}$ The renin-angiotensin system has been implicated in the progression of atherosclerosis in animal models.$^{86,88–90}$ and recent clinical evidence from the Heart Outcomes Prevention Evaluation trial has shown reduced overall cardiac morbidity and mortality in normotensive patients at higher risk for cardiovascular disease who were treated with an ACEI.$^{91}$ Although the mechanisms involved are not yet clear, there is abundant evidence that the cellular actions of AngII are pro-inflammatory and potentially injurious to the blood vessel. Reports have shown that ACEIs and AT$_1$ antagonists are capable of lessening lesion formation in atherosclerosis.$^{92}$ Others have shown NAD(P)H oxidase activation in hypercholesterolemia and atherosclerosis.$^{93–95}$ More recently, Daugherty et al$^{51}$ showed that subpressor doses of AngII could promote lesion formation and aneurysm formation in apo E /- mice; however, the mechanisms involved were not described. Adventitial proliferation was activated in those aortic regions where aneurysms were present. Thus, it is tempting to speculate that adventitial oxidase may be an early signaling agent in this response. Two recent reports showed that a deficiency in an
essential component of the leukocyte-related NAD(P)H oxidase had no effect on atherosclerotic lesion formation in apo E -/- mice under nonhypertensive conditions. However, one more recent study indicated that p47phox deletion diminished progression of atherosclerosis in apo E -/- mice in areas of the mouse aorta with lower degrees of lesion formation. Thus, studies are required to carefully examine the upregulation of oxidase isoforms during the development of atherosclerosis from early to late stages. We also believe that AngII, acting by way of stimulation of NAD(P)H oxidase, may be necessary before the oxidase can be fully involved in atherosclerosis, and thus, models that exhibit renin-angiotensin system activation should be considered. Such studies may provide the elusive link between hypertension and an increased propensity for atherosclerosis and clarify whether adventitial cells and their oxidases are involved directly or indirectly in lesion development.

Prototype NADPH Oxidase in the Phagocyte
Phagocyte NADPH oxidase (or respiratory burst oxidase) is a well-characterized ROS-generating system that catalyzes the one-electron reduction of oxygen to O$_2^·$. It is a multicomponent enzyme complex that includes the 2 membrane-spanning polypeptide subunits p22$^{phox}$ and gp91$^{phox}$ that are associated with the membrane cytoskeleton (which together comprise flavocytochrome b$_{558}$) and 4 cytoplasmic polypeptide subunits, p47$^{phox}$, p67$^{phox}$, p40$^{phox}$, and the cytosolic guanine nucleotide–binding protein p21$^{ras}$, a member of the Ras family of peptides. Exposure of the cell to a variety of agonists induces phosphorylation of cytosolic components and association of cytosolic and membrane-associated components and activates normally dormant oxidase.

NAD(P)H Oxidases in VSMCs, Endothelial Cells, and Adventitial Fibroblasts
Numerous reports have demonstrated NAD(P)H oxidase(s) in VSMCs, endothelial cells, and fibroblasts. Before these more recent discoveries, the most widely studied sources of O$_2^·$ were NAD(P)H oxidase in white cells and xanthine oxidase in endothelial cells, which plays an important role in impaired EDR of aortas from hypercholesterolemic rabbits. Ushio-Fukai et al. extensively described an NAD(P)H oxidase that interferes with relaxation in VSMCs of the rat aorta, which express the mRNA for 1 of the cytochrome b$_{558}$ subunits found in phagocyte membranes. They also found a homologue of gp91$^{phox}$, called nox1, in smooth muscle that participates in O$_2^·$ production and serum-dependent growth. In cultured rat aortic SMCs, NAD(P)H oxidase O$_2^·$ activity is stimulated by AngII and is involved in the hypertensive response as well as in the development of hypertension. They used the cloning for p22$^{phox}$, which is reportedly present in VSMCs and fibroblasts, to show that this subunit binds nox1 or nox4. The other major cytosolic component of phagocyte NAD(P)H oxidase, p67$^{phox}$, has been detected in fibroblasts and has been found in both cellular types and has been described as functionally required by the enzyme in aortic VSMCs. In rat VSMCs, p22$^{phox}$ cDNA bears a high homology to the human neutrophil oxidase sequence.

Cytosolic p47$^{phox}$ is reportedly present in VSMCs and fibroblasts, and there is evidence that it is an essential component in VSMCs, although it is not yet clear whether this subunit binds nox1 or nox4. The other major cytosolic component of phagocyte NAD(P)H oxidase, p67$^{phox}$, has been detected in fibroblasts and has been found in membranes of thrombin-stimulated human aortic VSMCs and transfected of NIH 3T3 fibroblasts with a dominant-negative allele of Rac1 decreased ROS production.

Activation and Kinetics
These structural differences between oxidases likely lead to differences in biochemical behavior as well as enzymatic activity. Phagocyte NAD(P)H oxidase does not produce O$_2^·$ under basal conditions; however, VSMCs and fibroblasts exhibit low basal O$_2^·$ activity. Early data indicated that vascular oxidase (unlike neutrophil oxidase) is assembled and...

Structural Differences in NAD(P)H Oxidases Present in VSMCs Versus Fibroblasts
Early studies of phagocyte NAD(P)H oxidase formed the basis of comparison for vascular isozymes. Preliminary evidence suggests that the fibroblast enzyme resembles the phagocytic enzyme, whereas the VSMC oxidase varies significantly in its gp91$^{phox}$ homologues. VSMCs and fibroblasts have NAD(P)H oxidases that both appear to be associated with the plasma membrane. Whereas fibroblast expression of gp91$^{phox}$ (nox2) has been reported in many species, much of the evidence suggests it is weakly expressed at the protein level in VSMCs; moreover, very low levels of mRNA were detected in rat aortic VSMCs, the focus of most studies. In contrast, expression of gp91$^{phox}$ homologues nox1 (mitogenic oxidase) and nox4 has been detected at high levels in human fibroblasts and fibroblasts of other species, much of the evidence suggests it is weakly expressed at the protein level in VSMCs; moreover, very low levels of mRNA were detected in rat aortic VSMCs, the focus of most studies. In contrast, expression of gp91$^{phox}$ homologues nox1 (mitogenic oxidase) and nox4 has been detected at high levels in human fibroblasts and fibroblasts of other species, much of the evidence suggests it is weakly expressed at the protein level in VSMCs; moreover, very low levels of mRNA were detected in rat aortic VSMCs, the focus of most studies.

We partially cloned p67$^{phox}$ and found a very high degree of homology with human neutrophil p67$^{phox}$ and reported its potent transcriptional induction by AngII. Recent partial cloning of gp91$^{phox}$ in rabbit adventitial fibroblasts showed that it is highly homologous to the human neutrophil gp91$^{phox}$, suggesting a marked similarity between fibroblast and endothelial oxidase isoforms.
Evidence for the Involvement of Vascular NAD(P)H Oxidase–Derived ROS in Cell Signaling Leading to Cellular Growth

The interaction of ROS at the whole-cell level appears to take place at growth factor receptors. Epidermal growth factor receptor (EGF-R) and platelet-derived growth factor receptor are both transactivated by AngII, a process mediated by ROS derived from NAD(P)H oxidases. The resulting tyrosine phosphorylation generally leads to activation of src homology complex–growth factor receptor–bound protein 2–son of sevenless complex (Shc-Grb2-Sos) that activates ras, leading to downstream activation of mitogen-activated protein kinases (MAPKs) and transcription factors. Some of the key redox-sensitive kinases playing a role in this cascade are ERK1/2, c-Jun N-terminal kinases, big MAPK, and p38 MAPK, which appear to converge at the site of activation of the Akt/protein kinase B pathway and result in cellular hypertrophy. NAD(P)H oxidases have been most clearly implicated in the activation of p38 MAPK and JNK. In response to oxidative stress, VSMCs also secrete factors that promote ERK1/2 activation and growth. Cyclin A (CypA) is an important oxidative stress–induced factor, which is secreted by VSMCs and fibroblasts during oxidative stress, and injured coronary arteries secrete CypA during the first week after injury, concomitant with ontinual proliferation (for a comprehensive review on signaling, see Griendling et al).}

Does Divergent Signaling in Fibroblasts Lead to Cell Proliferation?

Whereas ROS stimulate MAPK activation, gene transcription, and primarily hypertrophy in VSMCs, in fibroblasts a mitogenic response appears to prevail. In vascular adventitial fibroblasts per se, the involvement of specific redox-sensitive signaling pathways leading to cell proliferation is less clear. However, a few key studies provide insight into these mechanisms, and we are able to glean important information from a variety of other fibroblast preparations. EGFR internalization, ubiquitination, and thus, downregulation are inhibited by H2O2 in human 3T3 fibroblasts. AngII activates p21WAF1/Cip1/Cip2, and this in turn activates Ras and Raf, a downstream effector of p21WAF1. AngII is known to activate p21WAF1, but to our knowledge, this has not been confirmed in fibroblasts. In embryonic fibroblasts, ROS activate Fyn, which phosphorylates JAK2, and in turn activates Ras and Raf, which are both implicated in cell cycle progression and fibroblast mitogenesis. Although ROS are suggested to be involved in transmodulation of fibroblasts to myofibroblasts, the pathways involved in this process are not known (Figure 2). Cytokine-induced c-myc gene expression in human dermal fibroblasts is mediated by the redox-sensitive nuclear factor-xB, which may explain how adventitial inflammation can activate fibroblast proliferation. Still, there appears to be a high degree of overlap in the signal-transducing pathways of VSMCs and fibroblasts, which ultimately promote hypertrophy and mitogenesis, respectively. Thus, VSMCs and fibroblasts likely vary widely in their ability to activate cell cycle arrest inhibitors such as p27Kip, which is induced by AngII and activated by ROS. One very enlightening study examining the ability of NO to increase p21WAF1/Cip1/Waf1 kinase...
inhibitor levels shows clear divergence in the pathways leading to S-phase arrest.135 Currently, the major apparent differences between fibroblasts and VSMCs are at the level of nox isoforms, perhaps suggesting a link between the isoform, its subcellular distribution, and function. In fact, it has been predicted that nox4 is confined to the endoplasmic reticulum and is involved in cell quiescence,114 suggesting that its unique expression in VSMCs (versus other nox isoforms in fibroblasts) could predispose VSMCs to pathways leading to cell cycle inhibition, including p27Kip and Akt/protein kinase B expression. Because upstream signaling agents converging at the various MAPKs appear to be markedly similar, there is likely a unique combination of transcription factors and early-response genes that allows fibroblasts to enter the cell cycle and proliferate, whereas VSMCs do not. Inasmuch as p53 tumor suppressor protein has recently been demonstrated to be irreversible in senescent cells,136 one question that remains to be addressed is whether p53 is induced by ROS and more tightly coupled and irreversible in VSMCs. It is also likely that the various nox isoforms vary significantly in their ROS-producing capacities. Thus, the sensitivities of colocalized kinases to ROS derived from these isoforms will likely be critical in whether or not a particular pathway is activated.

Conclusions
The adventitia is increasingly being considered a highly active segment of vascular tissue that contributes to a variety of disease pathologies, including atherosclerosis and hypertension. Sensitivity of adventitial fibroblasts to local stimuli involved in the production of NAD(P)H oxidase-derived ROS could affect the function of the entire vascular wall, including endothelial dysfunction and the ability of vascular cells to proliferate. Many questions remain as to the specific pathways initiated by NAD(P)H oxidase, which diverge in adventitial cells and VSMCs, leading to either hyperplasia or hypertrophy.

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