Many cells that comprise the vasculature generate reactive oxygen species (ROS). Conventional thought has generally regarded these elementary molecules as harmful to the vasculature, leading to such pathological processes as hypertension, restenosis, and atherosclerosis. However, controlled clinical trials have failed to show a consistent benefit of antioxidants on atherosclerotic disease and its sequelae. Although a number of factors may contribute to this lack of efficacy, one intriguing possibility is that ROS through their many effects on vascular cells play both a physiological and pathophysiological role in vascular homeostasis. The purpose of this review is to summarize the varied effects that ROS have on vascular smooth muscle and endothelial cell growth, death, and survival. The pertinent redox-sensitive targets of ROS in these cells that mediate these effects will be discussed. Finally, a hypothesis for the mechanism(s) by which ROS result in diverse phenotypes in endothelial and smooth muscle cells will be presented.

Smooth Muscle Cells

Vascular smooth muscle cell (SMC) accumulation and hypertrophy are characteristic of atherosclerotic, restenotic, and hypertensive vascular diseases (reviewed in Reference 4). The net balance between proliferation and apoptosis determines the extent of SMC growth.
Proliferation

SMCs respond to growth factor stimulation with intracellular production of ROS. Such ligands include those acting via tyrosine kinase receptors such as platelet-derived growth factor (PDGF) and G protein–coupled receptors such as phenylephrine and thrombin. For instance, PDGF, a mitogen implicated in atherogenesis, stimulates the production of H$_2$O$_2$ in vascular SMCs and leads to SMC growth. Suppression of the PDGF-stimulated rise in H$_2$O$_2$ blunts this proliferative response. Similarly, thrombin stimulates H$_2$O$_2$ and superoxide production in SMCs. Suppression of these ROS by treatment with catalase or superoxide dismutase inhibits thrombin-induced mitogenesis. Finally, stimulation of SMCs with phenylephrine leads to induction of H$_2$O$_2$, suppression of which inhibits phenylephrine-induced proliferation.

A role for ROS, especially H$_2$O$_2$, in SMC growth is further supported by the finding that exogenous H$_2$O$_2$ or chemical agents that generate ROS induce tyrosine phosphorylation of mitogen-activated protein kinases, and cell growth. Taken together, these studies strongly suggest that ROS, and H$_2$O$_2$ in particular, mediate the proliferative phenotype in vascular SMCs.

Survival

In parallel to their important role in SMC proliferation, ROS have also been shown to be necessary for SMC survival. Suppression of endogenous intracellular H$_2$O$_2$, through overexpression of catalase or treatment with membrane-permeable antioxidants, not only inhibits proliferation but also promotes apoptosis in SMCs. Thus, ROS, and H$_2$O$_2$ in particular, act as signaling intermediaries in antiapoptotic pathways in vascular SMCs.

Hypertrophy

Angiotensin II (Ang II), a proinflammatory mediator implicated in atherosclerosis, restenosis, and hypertension, leads to the hypertrophic response in SMCs via the production of both superoxide and H$_2$O$_2$ and activation of p38 MAPK. Suppression of ROS inhibits Ang II–induced hypertrophy. Thus, ROS have also been linked with Ang II–induced pathological SMC hypertrophy.

Apoptosis and Growth Arrest

ROS, in addition to acting as growth-promoting signaling molecules, can also suppress growth and/or lead to programmed cell death in SMCs. Overexpression of the tumor suppressor gene p53 leads to an increase in ROS in SMCs, growth inhibition, and/or apoptosis. Furthermore, suppression of p53-induced ROS abrogates p53-induced apoptosis. Thus, in the context of p53-regulated cell-cycle progression, ROS are negative regulators of vascular SMC growth and survival. The physiological significance of these findings is supported by studies showing that p53 is an important endogenous regulator of SMC growth, and that inactivation of p53 is strongly associated with pathological SMC proliferation in human restenotic lesions.

Other studies using exogenously generated oxidants have similarly reported that ROS lead to cell death of SMCs. Interestingly, some of these studies have shown that exposure of SMCs to relatively low levels of oxidant stress for short periods promotes growth, whereas prolonged exposure to higher concentrations leads to cell death. Moreover, the species of oxidant added was important in determining the fate of the cell: superoxide resulted in cell growth whereas H$_2$O$_2$ led to cell death.

Endothelial Cells

Endothelial cell (EC) growth, death, and function are important determinants of vascular homeostasis.

Apoptosis

Although a causative role for EC apoptosis in the pathogenesis of vascular diseases has not been proven, mounting evidence shows that EC loss is a prominent feature of human atherosclerosis. Apoptotic ECs become procoagulant. In addition, the importance of EC apoptosis in atherogenesis and the role of...
ROS in this process are supported by studies showing that many risk factors for vascular disease promote apoptotic death of ECs through redox-dependent signaling. These include oxidized LDL and lipoprotein(a),22–24 high glucose and insulin,25,26 and Ang II.27,28 Moreover, ROS have been implicated in EC anoikis.29 Thus, ROS may play an important role in mediating apoptotic death in ECs that lose their interaction with the subendothelial matrix as seen at sites of atherosclerosis and those exposed to proatherogenic factors.

Survival
Although the role of ROS in promoting endothelial dysfunction and death has been well studied, the role of endogenously generated ROS in EC survival is relatively unknown. In nonvascular cells, superoxide production regulated by the small GTPase Rac1 protects against apoptosis.30 Similarly, recent evidence from our laboratory also points toward a crucial role for ROS generated by a Rac1-regulated oxidase in suppressing EC death via activation of nuclear factor-κB, whereas ROS produced independent of Rac1 promote EC apoptosis.31 Thus, by most accounts, endothelial production of ROS leads to cell death or promotes dysfunction. On the other hand, ROS specifically produced by a Rac1-regulated oxidase appear to prevent apoptosis of ECs.

The Table summarizes the role of ROS in SMC and EC growth, apoptosis, and survival.

| Sources of ROS in SMCs and ECs: The Importance of an NAD(P)H Oxidase |
|-----------------------------------------------|---------------------------|
| The observation that SMCs and ECs are capable of producing ROS has spurred a great deal of interest in identifying the enzymatic source(s) of these oxidants. A variety of cellular enzymes are potential candidates, including those involved in arachidonic acid metabolism, microsomal cytochrome P-450, xanthine oxidase, and mitochondrial electron transport. Arguably the most exciting recent discovery in this area is that ECs and SMCs possess an NAD(P)H oxidase activity analogous to the multicomponent phagocytic NADPH oxidase. The functional characteristics of this oxidase and its importance in cardiovascular biology and disease are covered in much greater detail in another review in this thematic series.32 Suffice it to say that many, although not all, components of this oxidase have been identified at the RNA or protein levels in SMCs and ECs.3,7,33–37 In addition, Rac1, a small GTPase that is an essential regulatory component of the phagocytic oxidase,38 is ubiquitously expressed.

Potential Targets of ROS in ECs and SMCs
The list of intracellular targets of ROS is growing rapidly. A detailed review of this list is beyond the scope of this article. However, within the context of vascular cell growth and apoptosis, certain names merit particular attention (Figure).

Summary of References in This Review Supporting Different Roles of ROS in SMC and EC Growth, Apoptosis, and Survival

<table>
<thead>
<tr>
<th>Growth</th>
<th>Apoptosis</th>
<th>Survival</th>
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<tbody>
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<td>5–10, 14, 15</td>
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<tr>
<td>ECs</td>
<td>...</td>
<td>22–29, 31</td>
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Extracellular Signal–Regulated Kinases
The mitogen-activated protein kinase (MAPK) family, also known as extracellular signal–regulated kinases (ERKs), is activated by exogenous H2O2 and by endogenously generated ROS in SMCs stimulated with growth factors.5 ERKs are important mediators of proliferation. Activation of ERKs has also been implicated in vascular endothelial growth factor (VEGF)–induced EC survival.39

Stress-Activated Protein Kinases
Kinases belonging to the stress-activated protein kinase (SAPK) family, which include c-Jun N-terminal kinases (JNKs) and p38 MAPK, are also sensitive to redox modulation (reviewed in Reference 40). Members of the Rho family of small GTPases including Rac1 regulate these kinases.40 In contrast to ERKs, JNKs and their downstream target c-Jun, have been implicated in H2O2 and other stress-induced apoptosis of ECs.51,52 Moreover, p38 MAPK has been implicated in EC upregulation of intercellular adhesion molecule-1 and, therefore, endothelial dysfunction.43 In SMCs, redox-sensitive activation of p38 MAPK mediates Ang II–induced hypertrophy14 and has also been implicated in SMC migration.44

Nuclear Factor-κB (NF-κB)
Activation of the transcription factor NF-κB has been associated with EC dysfunction and vascular inflammation.45 NF-κB–mediated transcription is also important in cell survival (reviewed in Reference 46). The activation of NF-κB by ROS, specifically ROS generated by a Rac1-regulated NAD(P)H oxidase, has been shown in HeLa cells.47 In SMCs, constitutive activation of NF-κB has been reported to be essential for proliferation.48 In addition, Ang II–induced effects on SMCs may also be mediated via NF-κB.49 In ECs, NF-κB is a prime target for ROS, and its activation has been linked to EC dysfunction (reviewed in Reference 45) and survival.31,50–53

Akt Kinase
Akt is a kinase, which lies downstream of phosphoinositide 3-kinase (PI 3-kinase) (PI 3-kinase), and is involved in antiapoptotic signaling (reviewed in Reference 54). It is regulated by ROS in Ang II–stimulated SMCs.50 In ECs, activation of Akt has been linked to the protective effects of shear stress56 and VEGF-induced growth and survival.57–59 Rac1 is a target for the products of PI 3-kinase,60 implicating a Rac1-regulated, NAD(P)H-dependent oxidase in the signaling pathways involving Akt in ECs and SMCs.

Caspases
Caspases are cysteine proteases that execute the apoptotic message. Caspasess are sensitive to redox changes in the cell (reviewed in Reference 61). Specifically, in ECs, processing and activity of the downstream caspase-3 in response to cell detachment62 or tumor necrosis factor (TNF) stimulation63 are regulated by ROS.

It is worth emphasizing that many of the signaling proteins mentioned above that are sensitive to the redox state of the cell may not be direct targets of ROS. In fact, it is very likely that one or more intermediary proteins are involved. Tyrosine phosphatases are prime candidates for such intermediaries. Such phosphatases all have redox-sensitive cysteine residues in their active sites,62 which are essential for their biological activity.63 The
generally accepted paradigm is that an increase in intracellular ROS by inhibiting tyrosine phosphatase activity transiently tips the balance toward tyrosine kinases that then leads to phosphorylation of their cellular targets, such as ERKs and SAPks.

The Answer to the Paradox: What, Where, How, and How Much?
The studies reviewed above show that ROS generated within vascular SMCs and ECs can either induce cell growth or arrest or promote survival or death, thereby leading to vascular dysfunction or acting as mediators of physiological vascular function. Although recently appreciated in vascular biology, such apparently paradoxical roles of ROS have been recognized in other fields of biology. The explanation to this paradox has been hinted at in the studies reviewed above and is probably a combination of the following factors:

1. What: The Species of Oxidant(s) Produced and the Proportion of Different Oxidant Species. Certain highly reactive oxidant species such as OH and peroxynitrite are more cytotoxic than others (reviewed in Reference 64). Moreover, ROS have differential effects on cellular targets such as ERKs10 and on cell growth.14 Thus, the redox milieu of the cell including its iron content and expression of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase, which play an important part in determining the species and amounts of ROS, is probably a key factor in determining the response of a cell to ROS production.

2. Where: The Subcellular Localization of the ROS. Similar to nitric oxide synthases, oxidoreductases are spatially distributed in a selective fashion,65 effectively controlling access of targets for ROS produced from different intracellular sources.

3. How: The Kinetics of ROS Production. The kinetics of oxidant production could differentially activate and/or inhibit targets such as transcription factors, resulting in a host of cellular responses. Such differential activation of transcription factors allows the cell to use the same second messenger to elicit varied responses and is known to exist for other well-known second messengers systems such as Ca2+.66

4. How Much: The Amplitude of ROS Production. The quantities of ROS produced probably have a profound effect in determining the fate of the cell. This is supported by observations that activation of specific redox-sensitive kinases such as ERKs and p38 MAPK in SMCs is very dependent on the concentration of ROS.5,14

Summary
The experimental data to date suggest potential conflicting roles of oxidants in the genesis of vascular disorders. This presents a challenge to scientists and clinicians alike. It is imperative that future efforts be directed toward better defining and characterizing the signaling pathways regulated by ROS in vascular cells. Such efforts will likely yield new molecular targets and ultimately more effective therapies for preventing or ameliorating vascular diseases such as atherosclerosis, restenosis, and hypertension, through fine modulation of ROS-regulated signaling.

Acknowledgments
This review was supported by The Johns Hopkins University Clinician Scientist Award, The W.W. Smith Charitable Trust, the Mid-Atlantic American Heart Association, the Bernard Foundation, and the Abraham and Virginia Weiss Endowment. I thank C.J. Lowenstein for constructive criticism of this manuscript. I also thank my collaborators P.J. Goldschmidt-Clermont and T. Finkel and all the members of my laboratory for helpful comments and discussions.

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Circ Res. 2000;87:179-183
doi: 10.1161/01.RES.87.3.179

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:
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