Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression

Charles D. Searles
Division of Cardiology, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia

Searles, Charles D. Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression. Am J Physiol Cell Physiol 291: C803–C816, 2006. First published May 31, 2005; doi:10.1152/ajpcell.00457.2005.—The ability of the endothelium to produce nitric oxide is essential to maintenance of vascular homeostasis; disturbance of this ability is a major contributor to the pathogenesis of vascular disease. In vivo studies have demonstrated that expression of endothelial nitric oxide synthase (eNOS) is vital to endothelial function and have led to the understanding that eNOS expression is subject to modest but significant degrees of regulation. Subsequently, numerous physiological and pathophysiological stimuli have been identified that modulate eNOS expression via mechanisms that alter steady-state eNOS mRNA levels. These mechanisms involve changes in the rate of eNOS gene transcription (transcriptional regulation) and alteration of eNOS mRNA processing and stability (posttranscriptional regulation). In cultured endothelial cells, shear stress, transforming growth factor-β, lysophosphatidylcholine, cell growth, oxidized linoleic acid, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, and hydrogen peroxide have been shown to increase eNOS expression. In contrast, tumor necrosis factor-α, hypoxia, lipopolysaccharide, thrombin, and oxidized LDL can decrease eNOS mRNA levels. For many of these stimuli, both transcriptional and posttranscriptional mechanisms contribute to regulation of eNOS expression. Recent studies have begun to further define signaling pathways responsible for changes in eNOS expression and have characterized cis- and trans-acting regulatory elements. In addition, a role has been identified for epigenetic control of eNOS mRNA levels. This review will discuss transcriptional and posttranscriptional regulation of eNOS with emphasis on the molecular mechanisms that have been identified for these processes.

eNOS AND VASCULAR HOMEOSTASIS

During the past two decades, it has become clear that the endothelium plays a predominant role in modulating many aspects of vascular homeostasis. One of these roles is regulation of vasomotor tone (47). Although several vasoactive factors are produced by the endothelium, the principal and best characterized is endothelium-derived NO+, produced by endothelial nitric oxide synthase (eNOS). NO+ is a free radical gas that diffuses rapidly from the endothelium to adjacent cells. It produces vasodilation by activation of soluble guanylate cyclase, production of cGMP, and ultimately activation of a variety of downstream effectors, including cGMP-dependent protein kinase, cGMP-modulated ion channels, and phosphodiesterases (95). There are also other targets of NO+ and related molecules that are influenced by nitrosation and nitration reactions (131). These “related molecules” of NO+ include nitrosothiols and reactive oxides of NO+, such as peroxynitrite and other nitrosating species (28, 52, 56, 149).

Beyond its vasodilator effect, NO+ has a number of other beneficial roles in the vessel wall, including inhibition of vascular smooth muscle cell proliferation (39), reduction of platelet aggregation (1), reduction in expression of adhesion molecules (62), inhibition of lipid oxidation, and regulation of apoptosis (16). In regard to apoptosis, NO+ can alter the balance between cell proliferation and cell death because it can be either proapoptotic or antiapoptotic. High concentrations of NO+, generated by the inducible isoform of NOS found in cells of the immune system, promote apoptosis. On the other hand, appropriate amounts of NO+ generated by the endothelial isoform, suppress unwanted apoptotic cell death. In general, many of the functions of endothelial-derived NO+ seem to prevent or inhibit atherosclerosis. The bioavailability of NO+ in the vessel wall is dependent on multiple factors: the expression of eNOS (148), the presence of substrate and cofactors for eNOS (including L-arginine and tetrahydrobiopterin) (133), the phosphorylation status of eNOS (26, 32), and the presence of reactive oxygen species (ROS), which can inactivate NO+ (115).

When the various NOS isoforms were first identified, the brain and endothelial enzymes were termed “constitutive” isoforms, and the enzyme isolated from macrophages was termed inducible. Although it is appropriate to refer to eNOS as constitutive in that endothelial cells express a basal level of eNOS protein, it is now clear that steady-state levels of eNOS mRNA are subject to modest, but likely important, degrees of regulation. In the 1980s, Miller and Vanhoutte and colleagues...
(90, 91) showed that vessels chronically exposed to high flow could release more "endothelium-derived relaxing factor" than control vessels. In 1992, when Harrison and coworkers (101) first cloned bovine eNOS, it was found that exposure of cultured endothelial cells to shear stress increased both eNOS mRNA and protein levels. Soon after these results were published, other investigators confirmed that high blood flow in vivo increased eNOS expression. Increased expression of eNOS has been observed in surgically created animal models for high flow (9, 87, 98) and in exercise-training studies (61, 127, 129, 135, 151). Interestingly, enhanced eNOS expression in response to exercise training, which increases shear stress through increases in cardiac output (15, 138), may account for some of the clinical benefits of exercise in patients with vascular disease.

Subsequent to the early studies that established mechanical force-induced regulation of eNOS expression, other stimuli have been shown to modulate the expression of eNOS mRNA in vitro. Most of these stimuli have been implicated in vascular pathophysiology, and they include other mechanical forces, cell growth, cytokines, lipoproteins, growth factors, and oxidative stress (Table 1). In each of these cases, the changes in eNOS mRNA expression and the ability of the cells to produce NO’ are generally on the order of two- to threefold. Although this level of modulation may seem modest, it must be remembered that small changes in NO’ levels may have significant physiological effects. In the case of vascular relaxation, the dose response to NO’ is quite steep; miniscule increments in NO’ concentration may produce large changes in vascular tone (97, 124).

Recent studies have provided a more detailed understanding of the molecular mechanisms involved in the modulation of eNOS expression. Although eNOS mRNA steady-state levels may be influenced by changes in transcription, many studies have demonstrated the importance of posttranscriptional regulation in the response to numerous stimuli, particularly those that appear to have the greatest effect on eNOS protein levels. The reason for the importance of posttranscriptional regulation may relate to the finding that eNOS mRNA has a long half-life at baseline (10–35 h). Stable mRNA species are able to pool in the cytosol, and thus synthesis of the encoded proteins is likely to persist long after gene transcription has been repressed. Altering the half-life of stable transcripts in this pool may be the most rapid and efficient means of modulating steady-state mRNA levels and gene expression. This level of regulation would provide endothelial cells with flexibility to effect rapid phenotypic changes in response to different stimuli.

This review will focus on what has been learned about the molecular mechanisms involved in the transcriptional and posttranscriptional regulation of eNOS. It is not meant to provide an exhaustive discussion of physiological and pathophysiological stimuli that modulate eNOS expression. For this information, the reader is referred to several recent reviews (31, 34, 43, 76, 77, 136). In addition, posttranslational regulation of eNOS will not be discussed here, but this subject has also been recently reviewed (35, 43, 152).

### Table 1. Physiological and pathophysiological stimuli shown to regulate eNOS expression and their mode of regulation

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Transcriptional Regulation</th>
<th>Posttranscriptional Regulation</th>
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</thead>
<tbody>
<tr>
<td>Laminar shear stress</td>
<td>Increased transcription</td>
<td>Increased stability</td>
</tr>
<tr>
<td>Cyclic strain</td>
<td>Increased transcription</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cell growth</td>
<td>No effect</td>
<td>Increased stability</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Increased transcription</td>
<td>Increased stability</td>
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<tr>
<td>TGF-β</td>
<td>Increased transcription</td>
<td>Unknown</td>
</tr>
<tr>
<td>Lysophosphatidylcholine</td>
<td>Increased transcription</td>
<td>Unknown</td>
</tr>
<tr>
<td>Oxidized LDL</td>
<td>Decreased and increased transcription</td>
<td>Decreased stability</td>
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<tr>
<td>Oxidized linoleic acid</td>
<td>Increased transcription</td>
<td>Increased stability</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Decreased transcription</td>
<td>Decreased stability</td>
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<tr>
<td>LPS</td>
<td>No effect</td>
<td>Decreased stability</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Increased and decreased transcription</td>
<td>Decreased stability</td>
</tr>
<tr>
<td>Statins</td>
<td>No effect</td>
<td>Decreased stability</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Increased transcription</td>
<td>Increased stability</td>
</tr>
<tr>
<td>Protein kinase C</td>
<td>Unknown</td>
<td>Decreased stability</td>
</tr>
<tr>
<td>Thrombin</td>
<td>No effect</td>
<td>Decreased stability</td>
</tr>
<tr>
<td>Rho GTPase</td>
<td>Possibly increased transcription</td>
<td>Increased stability</td>
</tr>
<tr>
<td>VEGF</td>
<td>Increased transcription</td>
<td>Probable decreased stability</td>
</tr>
<tr>
<td>Histone deacetylase inhibition</td>
<td>Increased transcription</td>
<td>Decreased stability</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>Unknown</td>
<td>Decreased stability</td>
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eNOS, endothelial nitric oxide synthase.
A detailed dissection of the human eNOS proximal core promoter revealed two regulatory regions involved in basal eNOS transcription (58). Positive regulatory domain I (PRD I; -104 to -95 relative to transcription initiation) corresponded to a high-affinity Sp1 transcription factor recognition site and was found to bind Sp1 and two variants of Sp3. The other regulatory region, positive regulatory domain II (PRD II; -144 to -115), bound transcription factors Ets-1, Elf-1, YY1, Sp1, and MYC-associated zinc finger protein. Furthermore, there was evidence that basal eNOS promoter activity was dependent on functional interactions between the trans-acting factors that bind to these two regulatory domains. These data highlight the complex nature of the eNOS promoter and the fact that its regulation involves multiple cis and trans interactions (Fig. 1).

In addition to the cis-elements located closer to the transcription start site, a 269-nt enhancer sequence located -4907 to -4638 upstream of the transcription start site has been described for the human eNOS promoter (73). Several regions within this 269-nt sequence were important for transcription factor binding and full enhancer activity. Endothelial specificity of this enhancer appeared to be dependent on an interaction between this element in its native configuration and the eNOS promoter. EMSAs identified multiple protein complexes that are important for enhancer function, including Erg and other Ets-related factors, AP-2, Sp1-related factor, and myeloid zinc finger gene-like factors.

Several polymorphic variations of the eNOS gene have been identified, and many of these have been studied with regard to association with cardiovascular disease. Although some studies have demonstrated a relationship between a particular polymorphism and disease risk, many reports have been contradictory (146). One polymorphism that has been examined in greater detail with respect to regulation of eNOS expression is 27-nt (5'-GAAGTCTAGACCTGCTGCAGGGGTGAG-3') repeats in eNOS intron 4. Zhang et al. (156) discovered the biological existence of a 27-nt microRNA derived from the repeat sequence in eNOS intron 4. The expression of eNOS mRNA is largely restricted to the vascular endothelium, particularly that of medium to large sized arteries (148). Marsden and coworkers (14) performed transient transfections of human eNOS promoter/reporter constructs in a number of endothelial and nonendothelial cell types and found unusually high eNOS promoter activities in a variety of nonendothelial cells that did not express appreciable steady-state levels of eNOS mRNA. Because nonendothelial cells did not lack the necessary transcriptional machinery to produce eNOS mRNA, they reasoned that there must be another mechanism by which wild-type eNOS gene expression was suppressed in these cells. Using methylation-sensitive isoschizomer mapping and high-resolution sodium bisulfite genomic sequencing, they found that the eNOS promoter was more heavily methylated in nonendothelial cells than in endothelial cells. Therefore, methylation at CpG dinucleotides has been implicated in a number of distinct cellular processes, including transcriptional regulation, embryogenesis, regulation of chromatin structure, and cancer pathogenesis (54, 60). The differentially methylated region in the eNOS promoter localized to a region that included PRD I and PRD II (see above), and methylation of eNOS promoter-reporter regions was associated with a marked impairment of promoter activity in mammalian cells (14). Thus DNA methylation appears to play an important role in endothelial cell-specific expression of the human eNOS gene.

DNA methylation of promoters is often accompanied by histone modifications that render the chromatin effectively inaccessible to transcription factors (100, 112, 120). Marsden and coworkers (30) further examined the role of the chromatin

- **HRE**
- **ERG, AP-2**
- **Sp1, MZF**
- **NF-1**
- **SSRE**
- **Sma2d**
- **AP-1**
- **KLF2**
- **Ets1-1, Elf-1, YY1**
- **Sp1, MAZ, p53**
- **GATA2**
- **CACC**
- **Sp1, Sp3**
- **Invr4**
- **HRE**
- **PRD II**
- **PRD I**
- **27-nt repeats**

Fig. 1. Cis-acting elements within the human endothelial nitric oxide synthase (eNOS) promoter. Schematic organization is shown of transcription factor binding sites whose roles in modulation of eNOS transcription have been demonstrated experimentally. Also shown is intron 4, which contains the 27-nt repeat (5'-GAAGTCTAGACCTGCTGCAGGGGTGAG-3') polymorphism and has been shown to be a source of microRNA. HRE, hypoxia-inducible factor responsive element; PRD, positive regulatory domain; KLF2, Kruppel-like factor.
structure in eNOS mRNA expression and found a unique combination of specific histone modifications in nucleosomes that encompassed the eNOS core promoter and proximal downstream coding regions. Histone modifications at these regions, which had been previously demonstrated to exhibit cell-specific DNA methylation, were shown to be functionally relevant to eNOS expression. These findings support a model for cell-specific control of eNOS transcription: DNA methylation and chromatin structure regulate the endothelial cell-specific conformation of eNOS promoter. This cell-specific conformation then allows the formation of traditional cis/trans-regulatory complexes and the interaction of these promoter complexes with the basal transcription machinery (136).

**POSTTRANSCRIPTIONAL REGULATION: eNOS 3′-UTR AND cis-REGULATORY ELEMENTS**

In eukaryotes, the fate of newly transcribed RNA is subject to regulation at multiple levels before it becomes translated into protein. Posttranscriptional regulation may involve modification of the primary transcript, nucleocytoplasmic transport, subcellular localization, mRNA stability, and translation efficiency. Posttranscriptional control at these different levels is felt to be largely mediated by cis-acting RNA elements located in 5′- and 3′-mRNA untranslated regions (5′-UTRs and 3′-UTRs) (45, 107). Regulation is dependent on a combination of the primary and secondary structures of these elements and their recognition by trans-acting RNA binding proteins (80).

Studies directed at defining cis elements involved in posttranscriptional regulation of eNOS have focused on the 3′-UTR (Fig. 2). Sequence analysis of the human eNOS 3′-UTR revealed a high level of homology (66%) with the bovine eNOS 3′-UTR, particularly in the distal half of the 3′-UTR (66). In addition, there are three domains of 20 or more nucleotides that exhibit near identity (≥90%) between the human and bovine sequences (66), indicating the presence of evolutionarily conserved sequences that may have functional significance. Despite this, except for a region that contains two AUUUA motifs, the eNOS 3′-UTR does not appear to contain any other known cis-regulatory elements. The AUUUA motif has been commonly found in the 3′-UTR of labile transcripts (33, 92), but it does not appear to participate in modulation of eNOS mRNA half-life (123).

A 43-nt sequence located at the origin of the bovine 3′-UTR was shown to have a role in determining eNOS mRNA stability; a transfected chimeric eNOS mRNA construct was dramatically stabilized by deletion of this 43-nt sequence (123). Approximately 95 bases distal to the 43-nt sequence is a 25-nt UC-rich sequence that has also been shown to be important for modulating eNOS stability (2, 117). For human eNOS mRNA, a CU-rich 158-nucleotide sequence located in the medial portion of the 3′-UTR was identified as being important in regulating mRNA stability (66). Together, studies that have characterized cis-regulatory elements in the eNOS 3′-UTR suggest multiple RNA-protein interactions (Fig. 2), but more extensive analyses of these elements and their binding proteins are needed.

Another mechanism for the posttranscriptional regulation of eNOS has been proposed that relates to cell-specific eNOS expression. Robb et al. (111) identified an antisense mRNA that was complementary to human eNOS mRNA for 662 nt and appeared to downregulate eNOS expression posttranscriptionally in nonendothelial cell types. The antisense mRNA, called sONE, was derived from a transcription unit (NOS3AS) on the DNA strand opposite from eNOS. The two genes were oriented in a tail-to-tail configuration at human chromosome 7q36. The mRNA for sONE could be detected in a variety of human cell types, both in vivo and in vitro, but not in vascular endothelial cells. A murine sONE was also detected. Suppression of sONE expression in human vascular smooth muscle cells led to increased eNOS expression, whereas overexpression of sONE in human endothelial cells led to decreased eNOS expression. These findings suggest a model for cell-specific expression of eNOS that involves a functional interaction between the eNOS and NOS3AS genes at the postranscriptional level. Further studies are needed to determine how sONE expression is regulated in endothelial cells and its role in eNOS expression under various physiological and pathophysiological conditions.

**SPECIFIC TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL REGULATION OF eNOS EXPRESSION**

_Transforming growth factor-β1_. Transforming growth factor β1 (TGF-β1) is a 25-kDa homodimeric peptide that plays an important role in the pathogenesis of atherosclerosis, hypertensive vessel remodeling, and angiogenesis (106, 113, 118). In
bovine aortic endothelial cells (BAECs), TGF-β1 treatment increased eNOS mRNA in a time- and dose-dependent manner via augmenting transcription (53). Activity of a bovine eNOS promoter construct was increased fourfold by TGF-β1, and its cis-acting response element was mapped to a NF-1-like binding region −1014 upstream from the transcription start site (144). This region formed nucleoprotein complexes that were immunologically similar to CCAAT transcription factor/NF-1.

TGF-β1 also increased steady-state eNOS mRNA levels by 3.6-fold in human endothelial cells (119). Deletion analysis of the human eNOS promoter mapped the predominant TGF-β1 response element to a region extending from −1000 to −720 upstream from the transcription start site. In these studies, the corresponding TGF-β1 response element described for the bovine promoter (−1219 to −1191 in human) appeared to be relatively less important for TGF-β1 transactivation. The transcriptional Smad2 was found to interact with the −1000 to −720 regulatory element, despite the absence of a consensus Smad binding element. Furthermore, inhibition of Smad2 blocked TGF-β1-induced transactivation of the eNOS promoter. Thus TGF-β1 increases eNOS transcription via recruitment of multiple transcription factors (Smad2 and NF-1) to distinct cis-acting sequences, and there appears to be interspecies variability of this mechanism.

**Cyclosporine A, hydrogen peroxide, and lysophosphatidylcholine.** Immunosuppressive therapy with cyclosporine A (CsA) is associated with hypertension, but this agent has been shown to paradoxically increase NO production in vitro (83) and in vivo (10, 132). BAECs exposed to CsA had a threefold increase in the expression of eNOS mRNA, which was time dependent and maximal at 24 h (83). Nuclear run-on analysis of cells treated with CsA showed a 1.7-fold increase in the rate of eNOS transcription compared with control, which correlated with a CsA-induced twofold increase in activity of a human eNOS promoter-luciferase reporter construct. In these experiments, no specific CsA-responsive element was identified within the eNOS promoter, but the AP-1 binding site was felt to be involved (82). EMSAs of nuclear extracts from CsA-treated cells confirmed that the eNOS AP-1 sequence formed a nucleoprotein complex immunologically similar to AP-1, and some models of diabetes (50).

Increased expression of eNOS under conditions of increased ROS production is a recurrent theme in the studies discussed above. During the past several years, it has become clear that ROS produced in mammalian cells can serve physiological roles as signaling molecules and, when produced in excess, can participate in the initiation of disease. Increased expression of eNOS may represent a compensatory mechanism to preserve biological levels of NO in the face of increased ROS production. This compensatory mechanism may explain observations of increased eNOS levels and NO production in certain pathological states. These include hypertension (11), atherosclerosis (74), aging (143), some models of heart failure (8), and some models of diabetes (50).

Recently, Harrison and coworkers (68) described the endothelial function and blood pressure of a transgenic (Tg) mouse that overexpressed p22phox in smooth muscle. p22phox is an important catalytic subunit of NAD(P)H oxidases in vascular smooth muscle (67). The vessels of the Tg-p22^null^ mice produced excessive O$_2^-$ and H$_2$O$_2$, but their endothelium-dependent vasodilatation and blood pressure were normal. Tg-p22^null^ mice had a striking increase in eNOS expression and vascular NO$^+$ production and an increase in extracellular superoxide dismutase expression. It appears that chronic oxidative stress caused by excessive H$_2$O$_2$ production in vivo evoked a compensatory response involving increased eNOS expression, NO$^+$ production, and extracellular superoxide dismutase expression. These changes seemed to counterbalance increased ROS production and to help maintain normal vascular function and hemodynamics.

**Cell growth.** A potent stimulus for eNOS expression in vascular endothelial cells is growth, which has been described both in vitro (5) and in vivo (108). In proliferating BAECs, eNOS mRNA was increased four- to fivefold compared with cells several days after confluence (5). Nuclear run-on and actinomycin D transcriptional arrest studies indicated that growth-induced regulation of eNOS expression was entirely modulated by posttranscriptional mechanisms: there was no
difference in the rate of transcription, but the eNOS mRNA half-life was threefold greater in preconfluent compared with nonproliferating endothelial cells (123). In UV-crosslinking studies that examined RNA-protein interactions, an ~51-kDa protein was observed to bind to a 43-nt cis-element in the proximal portion of eNOS 3′-UTR (123). This ribonucleoprotein was detected predominantly in cytoplasmic extracts from nonproliferating endothelial cells, and its binding activity was shown to be inversely related to eNOS mRNA stability. Deletion of the 43-nt cis-element led to stabilization of transfected chimeric eNOS mRNA. Together, these findings suggested that the 51-kDa ribonucleoprotein was a destabilizing factor.

In subsequent studies, the destabilizing cytoplasmic 51-kDa RNA-protein complex was purified, and it was found that monomeric actin (G actin) was the predominant protein in the ribonucleoprotein complex (122). Therefore, the interaction of G actin with eNOS mRNA was associated with decreased mRNA stability. Formation of the ribonucleoprotein complex was inhibited by DNase I and thymosin β4, two proteins known to specifically bind and sequester G actin. In situ hybridization studies demonstrated colocalization of monomeric actin and eNOS mRNA in the cytosol of BAECs. The binding activity of actin to the eNOS 3′-UTR was linked to growth-related changes in endothelial cytoskeleton organization. These changes were quantified by the ratio of monomeric actin to filamentous actin (G-to-F actin ratio) in cells at different stages of cell growth. The G-to-F actin ratio in confluent cells was twofold greater (more G actin) than that in proliferating cells, and this correlated with increased binding of monomeric actin to eNOS mRNA in confluent cells. Furthermore, G actin binding was related to the cytosolic localization of eNOS mRNA. eNOS mRNA from proliferating cells had greater localization to cytoskeleton-bound polysomes compared with eNOS mRNA from confluent cells, and this was associated with less G actin binding.

For some proteins, cytoskeleton-mediated subcellular localization and targeting of their mRNAs play a significant role in determining efficient translation and proper protein localization (49). Because mRNA stability is often closely linked to translation, it was proposed that actin-mediated transport and localization of eNOS mRNA to either cytoskeletal-bound or free polysome fractions may contribute to growth-related differences in transcript stability. Because the endothelial actin cytoskeleton is subject to modulation by many physiological and pathophysiological stimuli, it is tempting to speculate that cytoskeleton organization may be part of a mechanistic link between cell growth and other stimuli that modulate eNOS mRNA stability. Interestingly, we also observed a dramatic decrease in G actin binding to the eNOS 3′-UTR in endothelial cells treated with H₂O₂ (122), and this correlated with a low G-to-F actin ratio in these cells.

TNF-α, LPS, hypercholesterolemia, and oxidized LDL. In 1993, Yoshizumi et al. (153) demonstrated that there was a dramatic decrease in steady-state levels of eNOS mRNA and protein in human umbilical vein endothelial cells (HUVECs) treated with the cytokine TNF-α (153). This finding was consistent with earlier work showing impaired endothelium-dependent vasorelaxation in isolated arteries treated with TNF-α (4). In nuclear run-on analysis of cells treated with TNF-α, there was no difference in the rate of eNOS transcription compared with untreated cells (153). However, TNF-α treatment resulted in a reduction of eNOS mRNA half-life from 48 h at baseline to 3 h.

TNF-α-induced destabilization of eNOS message has been observed by others (93), and RNA-protein interactions involved in this destabilization have been described. In BAECs, a 60-kDa cytoplasmic protein was identified that bound to a UC-rich, 25-nt cis-element in the 5′ half of the eNOS 3′-UTR (2, 117). Binding activity was increased by TNF-α treatment, but it was reduced by coinubcation with cerivastatin (42), an agent associated with increased stabilization of eNOS message. These data suggested that the 60-kDa protein was a destabilizing factor. Interestingly, the cis-element involved in TNF-α-induced destabilization of eNOS mRNA appears to be distinct from the growth-related cis-element (123), indicating the presence of multiple regulatory elements in the eNOS 3′-UTR.

In UV-crosslinking assays of protein extracts from HUVECs treated with TNF-α, three ribonucleoprotein complexes (RNPs) were identified when a riboprobe encoding the entire human eNOS 3′-UTR was used (66). These RNPs had molecular masses of 53, 56, and 66 kDa. The formation of the 53- and 56-kDa RNPs were upregulated by TNF-α, whereas the 66-kDa RNP was downregulated. The 56-kDa bound to a CU-rich cis-element in the medial portion of the eNOS 3′-UTR (located between 148 and 305 bases downstream from the stop codon) and was implicated as a trans-acting destabilizing factor based on the ability of exogenous RNA decay (overexpression of RNA encoding its binding site) to attenuate the effect of TNF-α on eNOS expression. The identity of this 56-kDa factor is unknown.

Recently, it has been demonstrated that TNF-α also has an effect on eNOS transcription. In a time- and dose-dependent fashion, TNF-α decreased the activity of a human eNOS promoter construct that had been transiently transfected into BAECs (3). Although the TNF-α-dependent inhibition of promoter activity appeared to involve the NF-κB cascade, the loci mediating the inhibition were mapped to two Sp1-binding sites positioned between −109 and −95 and −81 and −67 relative to the transcription start site. Interestingly, mutations of either locus greatly suppressed basal promoter activity, but only the upstream locus (−109 to −95) showed a decrease in Sp1/Sp3 binding activity in response to TNF-α. The link between reduction in Sp1 binding and NF-κB was unclear. Another study of TNF-α and eNOS transcription utilized reporter constructs that contained various truncations of the human eNOS promoter (99). Promoter activity was decreased in bovine lung microvessel endothelial cells treated with TNF-α (50 ng/ml); the TNF-α-responsive cis-acting elements were postulated to be located in a fragment of the promoter −494 to −166 relative to the transcription start site. Further analysis of this fragment suggested that TNF-α-response elements were −370CACC and −231GATA. EMSA analyses of nuclear extracts indicated that TNF-α increased binding of nuclear protein to the −370CACC probe and decreased binding to the −231GATA probe. Furthermore, EMSA supershift analyses indicated that the transcription factor Sp3 bound to the −370CACC site and GATA-4 bound to the −231GATA site.

Although these two studies both demonstrated that TNF-α suppresses eNOS promoter activity, they appear to be discrepant in that they emphasize the importance of different cis- and trans-acting elements. Although the reason for this discrepancy is not clear, the studies differed in the type of endothelial cells.
and the concentrations of TNF-α that were used. It is possible that gene-regulatory mechanisms in endothelial cells from diverse vascular beds differ in their response to TNF-α as well as other stimuli. Overall, it may be concluded that TNF-α downregulates eNOS expression and that this occurs through both transcriptional and posttranscriptional regulation.

LPS, a component of the cell walls of gram-negative bacterium and, like TNF-α, a mediator of inflammation, time- and dose-dependently decreased eNOS mRNA and protein levels in bovine coronary venular cells. This reduction appeared to be related to destabilization of the eNOS message (84). These findings were consistent with in vivo studies that described decreased eNOS expression in heart, lung, and aorta of rats injected with LPS (81).

Both LPS and Staphylococcus aureus endotoxin decreased eNOS mRNA levels in guinea pig pericardial tissues (7). The effect of these endotoxins appeared to be mediated through decreased mRNA stability, and endotoxin-induced downregulation of eNOS was prevented by aspirin treatment. With the use of gel-shift assays using riboprobes derived from the bovine eNOS 3′-UTR, a UC-rich region in the 5′ half of the 3′-UTR was shown to be the binding target for 51 and 60 kDa proteins from pericardial extracts. Binding of these proteins was increased by endotoxin treatment and reduced by coinubation with aspirin. The sizes of these proteins and their 3′-UTR binding region were similar to those observed with TNF-α (2). These data suggest a common pathway for destabilization of eNOS mRNA by endotoxins and possibly TNF-α.

A ribonucleoprotein complex similar to the one described above was observed in aortic segments of hypercholesterolemic rabbits (55). The aortic expression of eNOS mRNA and protein was reduced in hypercholesterolemic rabbits, and this was associated with diminished endothelium-dependent relaxation in these animals. Treatment of hypercholesterolemic rabbits with cerivastatin restored endothelium-dependent relaxation and eNOS expression. Subsequently, in aortic segments incubated ex vivo with actinomycin D, it was found that eNOS mRNA half-life was attenuated by hypercholesterolemia. The mechanism responsible for this attenuation perhaps involved an interaction between the UC-rich region described earlier and a 60 kDa cytoplasmic protein. Formation of the ribonucleoprotein complex was increased in aorta of hypercholesterolemia rabbits and decreased by cerivastatin treatment.

The 60 kDa eNOS ribonucleoprotein described above has been observed in endothelial cells from different species and in response to different stimuli. In addition, the UC-rich binding region is highly conserved between human, bovine, and rabbit eNOS 3′-UTRs (55). Together, these observations suggest that this ribonucleoprotein complex is part of a conserved pathway for eNOS mRNA destabilization. However, this work has shown only an association between ribonucleoprotein complex formation and destabilization and not causality. It remains to be proven whether the 60-kDa protein directly participates in modulation of eNOS mRNA half-life.

Whereas lysoPC is a potent activator of eNOS expression (see above), oxLDL has been shown to both upregulate and downregulate eNOS mRNA levels in vitro. Liao et al. (78) treated human saphenous vein endothelial cells with increasing concentrations of oxLDL (0–100 μg/ml) and found a time- and dose-dependent decrease in eNOS mRNA levels. When cells were treated with 50 μg/ml oxLDL for 72 h, eNOS mRNA levels were reduced by 3.2-fold, correlating with a decrease in eNOS activity. oxLDL (50 μg/ml) was found to shorten eNOS mRNA half-life from ~36 h at baseline to 10 h. Interestingly, nuclear run-off studies showed a biphasic pattern of transcriptional regulation of eNOS in response to oxLDL (50 μg/ml). In the first 6 h after treatment with oxLDL, there was a 25% decrease in the rate of eNOS transcription. At later time points (12 and 24 h), there was a 1.8- to 2.2-fold increase in transcription. The results of this study indicated that oxLDL regulated eNOS expression through a combination of early transcriptional inhibition and destabilization of the mRNA.

OxLDL (1–50 μg/ml) also dose-dependently decreased eNOS mRNA levels in BAECs (48). Similar to what had been shown previously (78), this effect was dependent on oxidation of LDL, as native LDL had a minimal effect on eNOS expression. Somewhat contradictory to these findings, Hirata et al. demonstrated that BAECs exposed to a low concentration of oxLDL (10 μg/ml) for 24 h had a 2.4-fold increase in eNOS mRNA levels; this was associated with an increase in eNOS protein (50a). When a higher concentration of oxLDL (100 μg/ml) was tested, eNOS mRNA levels were decreased vs. control. Increased eNOS expression was also observed in BAECs that had been treated with 13-hydroperoxy-octadecadienoate, an important component of oxLDL (110). This oxidized form of linoleic acid was shown to increase the rate of eNOS transcription and prolong mRNA half-life. Taken together, studies of the effects of oxLDL, lysoPC, and 13-hydroperoxy-octadecadienoate suggest that the regulation of eNOS expression in the atherosclerotic plaque is complex and likely involves multiple, opposing mechanisms.

Hypoxia. In response to hypoxia, systemic arteries dilate, allowing them to deliver more blood to peripheral tissues. In contrast, hypoxia induces vasoconstriction in the pulmonary circulation, leading to elevated pulmonary vascular resistance and pulmonary hypertension (94). To determine the role of eNOS expression in the vasoreactive responses of different vascular beds to hypoxia, numerous studies have been performed with different in vivo and in vitro models of hypoxia. As might be expected, the results of these studies have been varied; hypoxia has been associated with both upregulation and downregulation of eNOS expression. In addition, both transcriptional and posttranscriptional regulatory mechanisms have been described.

Rats exposed to hypoxia (10%) for 12 h, 48 h, or 7 days had decreased aortic levels of eNOS mRNA and protein compared with controls (139). The downregulation of eNOS expression was associated with attenuation of endothelium-dependent relaxation. In contrast, there were increased eNOS mRNA and protein levels in the lungs of rats exposed to 10% oxygen for 7 days and 3 wk (75, 128). Together, these data support the notion of regional variability in eNOS expression during systemic hypoxia.

In vivo studies of hypoxia have not been completely consistent with in vitro studies. BAECs exposed to 1% oxygen for up to 24 h had increased eNOS mRNA and protein levels; eNOS mRNA levels were twice basal levels after 6 h (6). This increase was attenuated when cells were cotreated with actinomycin D, suggesting that hypoxia induces eNOS expression via gene transcription. Indeed, hypoxia increased expression of a reporter gene that was driven by the human eNOS promoter. Hypoxia (1–10% oxygen) also increased eNOS mRNA expres-
sion in porcine aortic endothelial cells in a manner inversely proportional to oxygen tension (51). In contrast, hypoxic incubation (0% oxygen) of HUVECs decreased eNOS mRNA and protein expression. In these experiments, there was decreased eNOS transcription and reduced message stability (89). A similar effect of hypoxia on eNOS transcription, message stability, and expression was observed in human saphenous vein endothelial cells (3% oxygen) (70, 137), BAECs (3% oxygen), and bovine pulmonary endothelial cells (3% oxygen) (79). The reason for the variability of eNOS expression in these studies is unclear but may reflect differences in species, the vascular bed from which the endothelial cells were derived, and the duration and severity of hypoxic exposures.

There have been two studies that have examined the details of hypoxia-induced transcription of eNOS. In the first, a construct was used that contained a 3.5-kb fragment of the human eNOS promoter and a luciferase reporter gene (51). Hypoxia (6 h, 3% oxygen) increased luciferase expression in transfected ECV304 cells, and this was mimicked by various agents that modulate the redox state of the cell (increased NADPH-to-NADP ratio). The increase in promoter activity could be blocked by cotransfection of oligonucleotides containing either one of the two AP-1 binding sites of the human eNOS promoter, suggesting that hypoxia regulates eNOS expression via a redox-sensitive, AP-1-mediated transcriptional control. Coulet et al. (20) noted a biphasic response of eNOS mRNA expression to the hypoxia mimic desferrioxamine in HUVECs. The initial increase in eNOS mRNA levels was attributed to increased transcription, and hypoxia-responsive elements were mapped at position −5375 to −5366 relative to the transcription start site. This sequence contains two contiguous hypoxia-inducible factor binding sites that were subsequently found to be responsive to hypoxia-inducible factor 2.

Inhibition of histone deacetylation. NO is known to be a mediator of angiogenesis (96), and trichostatin A (TSA), an inhibitor of histone deacetylase (HDAC) activity, is able to prevent VEGF- or hypoxia-induced angiogenesis (25, 59). Human cells treated with TSA for 24 h had an 80% reduction in eNOS mRNA levels (114). Subsequently, TSA was shown to have a dual effect on eNOS expression: an induction of eNOS in HUVECs during early stages of TSA treatment (15 min to 4 h), followed by inhibition of eNOS expression after longer periods of treatment (37). A dual effect of TSA was also seen with different doses of the drug. A low dose of TSA (0.1 μg/ml) led to increased eNOS expression, but higher doses resulted in decreased eNOS levels.

There might be two separate mechanisms that account for the complex effects of HDAC inhibition on eNOS expression. The first mechanism entails activation of the eNOS promoter. TSA treatment was shown to enhance eNOS promoter activity (114), and this may be due to release of the transcription factor Sp1 repression by HDAC1 (37). Constitutive endothelial expression of eNOS is dependent on Sp1, and association of Sp1 with HDAC1 has been demonstrated to repress its activity in other cell lines (38, 58).

The second mechanism responsible for the effect of HDAC inhibition appears to be dominant and likely involves posttranscriptional regulation. TSA did not alter eNOS mRNA stability (114), but the downregulation of eNOS by TSA was blocked by cyclohexamide, suggesting that new protein synthesis was necessary for this mechanism (37). It has been hypothesized that TSA induces the de novo expression of an eNOS mRNA destabilizing factor, whose effect could not be detected in mRNA decay assays that used a transcription inhibitor (actinomycin D) (114). Although these data suggest a role for a destabilizing protein, it is possible that the destabilizing factor is sONE; TSA increased sONE mRNA levels in endothelial cells before the observed decrease in eNOS expression (111).

Regulation of eNOS expression by TSA has been shown to have physiological relevance. TSA treatment attenuated the NO-dependent relaxation of porcine coronary arteries, and it prevented tube formation in a human angiogenesis assay. These findings indicate that histone acetylation may not only be important in regulation of cell-specific expression of eNOS but also important in regulating endothelial function.

Laminar shear stress. One of the most potent regulators of eNOS mRNA expression is laminar shear stress, a stimulus that has been demonstrated to increase eNOS mRNA levels in vitro (101, 140, 141) and in vivo (87, 98, 127). In cultured BAECs, laminar shear increased eNOS mRNA levels by ninefold compared with nonsheared cells (147), an effect that was attributed to two separate mechanisms: a transient increase in eNOS transcription followed by a prolonged stabilization of eNOS mRNA (21). Davis et al. used nuclear run-on analysis to show increased transcription of eNOS during the first hour of exposure of BAECs to laminar shear stress (21). With longer periods of shear, the transcription rate returned to baseline, but there was a threefold increase in eNOS mRNA half-life. With both pharmacological inhibitors and dominant-negative adenoviral constructs, the tyrosine kinase cSrc was shown to be responsible for both increased transcription and stabilization of eNOS mRNA, but, at this point, the two signaling pathways diverged. Transcription of eNOS was mediated by the classical MAPK pathway Ras/Raf/MEK1/2 and ERK1/2. In contrast, laminar shear-induced stabilization of eNOS mRNA was found to be independent of this pathway.

The mechanism responsible for the laminar shear’s effect on eNOS transcription was further examined with chimeric human eNOS promoter-chloramphenicol acetyltransferase reporter constructs (23). Shear-induced activation of this promoter construct was dependent on cSrc and MEK1/2. Shear responsiveness was dependent on sequences between −1000 and −975 (relative to transcription initiation), a finding consistent with previous studies of the eNOS promoter (85, 130). Furthermore, it was determined that shear-induced activation of the eNOS promoter was due to binding of NF-κB subunits p50 and p65 to a shear-responsive element (GAGACC) −990 to −984 bp upstream from the eNOS transcription start site. Activation of the eNOS promoter by laminar shear could be prevented by either mutating the GAGACC binding site or cotransfection with a dominant-negative Ikβα.

Transactivation of the eNOS promoter by laminar shear appears to be part of a negative feedback loop that involves NO (44). In addition to activating NF-κB, laminar shear stress simultaneously increases enzymatic production of NO. The activation of NF-κB leads to translocation of p50/p65 heterodimers to the nucleus, where binding of these factors to the eNOS promoter leads to an increase in eNOS transcription, increasing eNOS protein levels and further augmenting NO production. The increased NO production ultimately leads to nitrosylation of p50 and inhibition of NF-κB, resulting in termination of eNOS transcription (44). This negative feedback
the ability of KLF2 to bind to this site was verified by gel-shift.

Recently, it was established that another transcription factor is involved in laminar shear-induced eNOS transcription. Dekker et al. (24) were the first to demonstrate increased expression of lung Krüppel-like factor (KLF2) in HUVECs exposed to sustained shear stress. These investigators showed that KLF2 mRNA is present in the endothelium of healthy human aortas, exclusively in areas of high flow. Subsequently, it was shown that adenoviral overexpression of KLF2 in HUVECs resulted in a dramatic induction of eNOS mRNA, protein, and enzyme activity (125). In deletion and mutational analyses of the human eNOS promoter, the region between −652 and −644 was identified as critical for KLF2-mediated transcription. This region contains a consensus KLF binding site, and the ability of KLF2 to bind to this site was verified by gel-shift studies.

The relationship between NF-κB-mediated and KLF2-mediated activation of the eNOS promoter in response to laminar shear is unclear, but some details of this relationship have been elucidated. In endothelial cells that contained adenovirally expressed KLF2 that were stimulated with IL-1β, there was no difference in p50, p65, IkB, and phosphorylated IkB levels compared with control cells. However, KLF2 was shown to inhibit the activity of p65, and this inhibition could be attenuated by overexpression of CREB binding protein (CBP)/p300, a cofactor critical for NF-κB activity (125). Furthermore, KLF2 was shown to interact with CBP/p300. Interestingly, in COS-7 cells containing a construct of the human eNOS promoter, transfected CBP/p300 increased eNOS promoter activity, and cotransfection of KLF2 and CBP/p300 augmented eNOS promoter activity to a greater degree than either factor alone. These data indicate that KLF2 may recruit p300 away from NF-κB, and, in a cooperative manner, KLF2 and p300 increase eNOS expression.

Although it seems that the increase in eNOS expression mediated by KLF2 would antagonize the increase mediated by NF-κB, these two mechanisms likely function at different times after the onset of the shear stress stimulus. Maximum activation of NF-κB appears to occur within 15–30 min after the onset of shear (23), whereas maximum induction of KLF2 expression occurs at 6 h (24). Thus it is possible that the NF-κB-related mechanism is transient and occurs immediately after the onset of the shear stress stimulus. In contrast, the KLF2-related mechanism, which also leads to attenuation of NF-κB activity, may be involved in upregulation of eNOS expression after prolonged exposure to laminar shear stress.

The potency of laminar shear in upregulating eNOS expression may be due to its ability to augment both transcription and mRNA stability. Compared with control, prolonged (>6 h) shear stress lengthens eNOS mRNA half-life more than threefold (21). Given the brief duration of the changes in transcription, it seems likely that mRNA stability is the primary factor in determining shear-induced upregulation of steady-state eNOS mRNA levels. Recently, our group (147) sought to further characterize the mechanism responsible for the effect of shear on eNOS mRNA stability and discovered a role for 3′ polyadenylation. In mammalian cells, 3′ poly(A) tails have been shown to regulate mRNA stability and translation (88, 116, 121); mRNAs with short 3′ poly(A) tails are less stable and less translationally active than those with long tails (36, 88, 145). Under basal conditions, bovine eNOS mRNA had a short 3′ poly(A) tail, consistent with the published sequences of the cloned bovine and human eNOS mRNAs (86, 101). In endothelial cells exposed to laminar shear stress, we found a dramatic increase in expression of eNOS transcripts with long 3′ poly(A) tails. Importantly, 3′ polyadenylation was dependent on the magnitude and duration of the shear stress stimulus. Long polyadenylated eNOS transcripts had prolonged half-lives (6 h in static cells vs. 18 h in sheared cells), and eNOS mRNA from sheared cells was more actively translated, consistent with the known role of the 3′ poly(A) tail in supporting translation.

Transcriptional activation was observed to be important for shear-induced polyadenylation but not completely necessary, suggesting some degree of interplay between transcriptional and posttranscriptional mechanisms. Furthermore, the time at which polyadenylated eNOS transcripts were detected after the onset of the shear stimulus (2 h) was consistent with the temporal relationship between shear-induced transcription and stabilization that had been described (21). These data support an analogy that was proposed to explain the ability of laminar shear to upregulate eNOS (21): the combination of transcriptional and posttranscriptional regulation is similar to a loading dose, followed by a maintenance dose of a medication administered to obtain rapid and sustained levels of a drug. On the basis of the finding that H₂O₂ and statin treatment also increased 3′ polyadenylation of eNOS, it is suspected that modulation of 3′ poly(A) tail length may be relevant to other stimuli that regulate endothelial gene expression.

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins). In both cell culture and animal studies, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (HMG CoA reductase; statins) have been shown to increase eNOS expression (22, 57, 69–71, 134). In animal models, the upregulation of eNOS by statin treatment resulted in decreased myocardial infarct and stroke size during ischemia (69, 150). In human saphenous vein endothelial cells, simvastatin and lovastatin both increased eNOS mRNA and protein levels in a time- and dose-dependent manner (70, 71). The mechanism responsible for this effect was found to be posttranscriptional; simvastatin treatment did not alter the rate of eNOS transcription but significantly prolonged of eNOS mRNA half-life as assessed by actinomycin D transcriptional arrest studies. Upregulation of eNOS expression by simvastatin was specific to inhibition of endothelial HMG CoA reductase because the effects of simvastatin were reversed by cotreatment with l-mevalonate, an isoprenoid that is a product of the enzymatic reaction catalyzed by HMG CoA reductase. In addition, these studies showed that simvastatin and lovastatin both counteracted the downregulation of eNOS expression by hypoxia and oxLDL (70, 71), two stimuli known to decrease eNOS mRNA stability (78, 79).

By inhibiting HMG CoA reductase, statins prevent the synthesis of isoprenoid intermediates in the cholesterol biosynthetic pathway (41). These intermediates serve as important lipid attachments for the posttranslational modification and activity of a variety of signaling proteins. Included in this group of proteins are members of the Rho GTPase family (142). Laufs and Liao (72) found that Rho negatively regulates eNOS expression in human endothelial cells; Rho inhibition...
increased eNOS expression, and Rho activation diminished eNOS expression. Furthermore, treatment of endothelial cells with statins decreased the geranylgeranylation, membrane translocation, and GTP binding activity of Rho. Therefore, statins appear to upregulate eNOS expression by blocking Rho geranylgeranylation. Interestingly, geranylgeranyl modification also appears to be involved in TSA-induced downregulation of eNOS expression. However, this latter mechanism is not dependent on Rho signaling (37).

The actin cytoskeleton is a downstream sensor of Rho GTPase, and each member of the Rho family serves specific functions in terms of cell shape, motility, secretion, and proliferation (46). Our group and others (69, 122) have demonstrated a role for endothelial cytoskeleton organization in posttranscriptional regulation of eNOS expression. Mice treated with a Rho inhibitor or the actin cytoskeleton disrupter cytochalasin D showed increased vascular eNOS expression and activity, and these changes were associated with a decrease in stroke size following ischemia (69). Similar neuroprotection was observed with statin treatment. Mutant mice lacking the gene for eNOS do not exhibit any neuroprotection after Rho inhibitor treatment, cytochalasin D treatment, or statin treatment. Therefore, changes in the endothelial actin cytoskeleton organization may represent the mechanistic basis for the effect of statins on eNOS expression. Alteration of Rho signaling by statins may lead to changes in endothelial actin cytoskeleton organization that affect the transport, localization, translation, and stability of eNOS mRNA. Furthermore, this mechanism that involves Rho signaling is likely not unique to statins; Rho/Rho kinase inhibition has been shown to reverse the downregulation of eNOS that occurs in response to thrombin (29) and hypoxia (137).

Recently, statins were shown to induce KLF2 expression in HUVECs in dose- and time-dependent manners (104, 126). KLF2 induction was observed within 8 h after statin treatment and was reversed by addition of either mevalonate or geranylgeranyl pyrophosphate, suggesting that the effect of statins was dependent on inhibition of cholesterol synthesis. In addition, the Rho pathway appeared to be involved in regulation of KLF2 expression. In siRNA-mediated knockdown studies, reduction in KLF2 mRNA in HUVECs was also associated with a 40% reduction in the basal level of eNOS protein (126). In cells that had been treated with mevastatin, upregulation of eNOS was completely lost when KLF2 expression had been knocked down. Similar findings were observed when another statin was used (104). These data strongly implicate KLF2 as a regulator of statin-mediated effects in the vascular endothelium. Because KLF2 has been shown induce eNOS promoter activity (125), transcriptional events may underlie the statin-related effect of KLF2, which is contradictory to previous reports describing an exclusive posttranscriptional mechanism for statin-induced eNOS expression (70, 71). However, the details of the mechanism involving statins and KLF2 are uncertain and require further investigation.

CONCLUSION

Since its initial characterization as a constitutively expressed enzyme, there have been numerous examples of physiological and pathophysiological stimuli that modulate eNOS expression. This phenomenon is not simply a cell culture oddity; in vivo studies have often confirmed in vitro data. Initial work on stimuli-induced changes in eNOS mRNA expression focused on whether regulation was transcriptional or posttranscriptional, but more recent work has sought to further define regulatory mechanisms, including cis-trans interactions and signaling cascades. Although much has been learned from these studies, many details of transcriptional and posttranscriptional eNOS regulation remain unclear. It has become apparent that regulation of eNOS expression associated with many
stimuli involves mechanisms that have both transcriptional and posttranscriptional components (Table 1, Fig. 3). In the future, it will be important to determine the significance of each component in steady-state eNOS mRNA expression.

GRANTS
Experiments performed in the author’s laboratory were supported by National Heart, Lung, and Blood Institute Grants HL-04062-01 and HL-077274-01.

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Invited Review


