Endothelial Nitric Oxide Synthase: A New Paradigm for Gene Regulation in the Injured Blood Vessel

Sharon C. Tai, G. Brett Robb, Philip A. Marsden

Abstract—Advances in our understanding of the molecular mechanisms involved in the constitutive and regulated expression of endothelial nitric oxide synthase (eNOS) mRNA expression present a new level of complexity to the study of endothelial gene regulation in health and disease. Recent studies highlight the contribution of both transcription and RNA stability to net steady-state mRNA levels of eNOS in vascular endothelium, introducing a new paradigm to gene regulation in the injured blood vessel. Constitutive eNOS expression is dependent on basal transcription machinery in the core promoter, involving positive and negative protein–protein and protein–DNA interactions. Chromatin-based mechanisms and epigenetic events also regulate expression of eNOS at the transcriptional level in a cell-restricted fashion. Although constitutively active, important physiological and pathophysiologic stimuli alter eNOS gene transcription rates. For instance, eNOS transcription rates increase in response to lysophosphatidylcholine, shear stress, and TGF-β, among others. Under basal conditions, eNOS mRNA is extremely stable. Surprisingly, posttranscriptional mechanisms have emerged as important regulatory pathways in the observed decreases in eNOS expression in some settings. In models of inflammation, proliferation/injury, oxidized low-density lipoprotein treatment, and hypoxia, eNOS mRNA destabilization plays a significant role in the rapid downregulation of eNOS mRNA levels. (Arterioscler Thromb Vasc Biol. 2004;24:405-412.)

Key Words: atherosclerosis ■ endothelium ■ gene regulation ■ mRNA stability ■ transcription

Animal models of altered endothelial nitric oxide synthase gene (eNOS) expression have emphasized the crucial role of this enzyme in a diverse array of vascular biology and pathobiology. Among others, eNOS/−/− mice have been associated with systemic and pulmonary hypertension,1–3 altered vascular remodeling,4,5 impaired angiogenesis,6,7 and perturbations in hemostasis.8 Recent studies have particularly underscored the important contribution of steady-state eNOS mRNA to the overall expression of the gene. In human atherosclerosis, for instance, eNOS mRNA expression was shown by in situ hybridization to be decreased in endothelial cells overlying advanced atheromatous plaques.9,10 A decrease in eNOS protein expression was also observed by immunohistochemistry.9 This downregulation of eNOS mRNA undoubtedly contributes to the muted endothelial nitric oxide (NO) production and defective endothelium-dependent vasorelaxation11 observed in diseased atherosclerotic vessels. It is important to reflect on the earlier important observations that impaired endothelium-dependent vasorelaxation can be observed even in face of adequate or enhanced vessel NO production.12 It is now appreciated that decreased endothelial eNOS mRNA and protein can be observed in diseased human blood vessels in which eNOS, inducible NOS (iNOS), and even neuronal (nNOS) are robustly expressed in the cells of the neointimal lesion.9

Regulated eNOS Expression

In recent years, in vivo models of eNOS expression and in vitro models of endothelial activation have provided a more in-depth understanding of the molecular mechanisms involved in the regulated expression of eNOS in response to various physiological and pathophysiologic stimuli. Numerous exogenous stimuli and conditions that are relevant to the pathobiology of vascular endothelium have been shown to alter eNOS expression through the modulation of steady-state eNOS mRNA (Table 1).13 Surprisingly, this regulation occurs at both the transcriptional and posttranscriptional levels and is the focus of this review. Therefore, eNOS mRNA levels do not correlate solely with gene transcription rates. Recent work in the posttranslational regulation of eNOS, although fundamental to eNOS biology, is not reviewed here. The readers are referred to recent reviews on this aspect of eNOS biology.14–16

Transcriptional Regulation

eNOS Core Promoter

The eNOS gene encodes a mRNA of 4052 nt and is present as a single copy in the haploid human genome. Sequence inspection of 5′-flanking regions revealed multiple potential cis-regulatory DNA sequences in the setting of a “TATA-less” promoter: Sp1, GATA, AP-1, NF-1, shear-stress re-
eNOS Expression in Response to Exogenous Stimuli

<table>
<thead>
<tr>
<th>Exogenous Stimulus</th>
<th>eNOS Steady State mRNA</th>
<th>eNOS mRNA Stability</th>
<th>eNOS Transcription</th>
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<tr>
<td>TNF-α</td>
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<td>↓</td>
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<tr>
<td>Hypoxia (acute)</td>
<td>↓ / ↑</td>
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<tr>
<td>Hypoxia (chronic)</td>
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<td>oxLDL (high levels)</td>
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<td>NC*</td>
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<td>LPC</td>
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<td>Laminar flow</td>
<td>↑ pending</td>
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<td>Perturbed flow</td>
<td>↓ pending</td>
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<tr>
<td>Cyclic mechanical stretch</td>
<td>↑</td>
<td>pending</td>
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<tr>
<td>Proliferation</td>
<td>↓ / ↑</td>
<td>↓</td>
<td>NC</td>
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<td>VEGF</td>
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<td>TGF-β</td>
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*NC, no change

A comparison of published in vivo models of eNOS expression highlights a potential role for chromatin-based or epigenetic mechanisms in the cell-specific regulation of the gene. Recent murine studies have found that an insertional transgene containing 5200 bp of the murine eNOS promoter (−5200/+/28 relative to transcription initiation) directed the expression of a β-galactosidase reporter in a fashion that recapitulated the known expression profile of eNOS mRNA and protein. This expression profile was found to be uniform across multiple founders and independent of integration site. In contrast, transgenic mice hemizygous for a promoter-reporter transgene containing 1600 bp of the human promoter (−1600/+/22) exhibited endothelial cell specificity and founder-specific expression patterns inconsistent with those observed for native eNOS. Interestingly, when this same promoter construct was targeted to the Hprt locus on the X chromosome (a known region of open chromatin), it was functionally able to direct reporter gene expression in a manner consistent with known eNOS expression profiles. Importantly, similar expression patterns were noted in multiple, independent founders. Taken together, these observations suggest that sequences between −5200 and −1600 of the eNOS 5′-flanking genomic region contain the requisite DNA elements that, in concert with −1600 to +22 promoter elements, appropriately directed cell-restricted transcription of eNOS in vivo.

The existence of a 269-bp enhancer sequence −4907 to −4368 nt upstream of the transcription start site in the human eNOS gene has also been noted. Although a classic enhancer in every sense, it was only in its native configuration in the context of human eNOS 5′-flanking sequences that this upstream enhancer demonstrated its endothelial specific property in the activation of a luciferase reporter. Nucleoprotein complexes containing MZF-like, Sp1-like, and Erg/Ets-like transcription factors were shown to form on this enhancer sequence. These data support the existence of a distal enhancer element that has the ability to direct the appropriate cell-specific expression pattern of the eNOS gene. Indeed, the transgenic promoter–reporter evidence indicates that the upstream enhancer region operative in the human promoter may also be functional in the murine promoter.

Using the high-resolution sodium bisulfite genomic sequencing method, it was recently suggested that the human eNOS proximal promoter was differentially methylated in expressing and nonexpressing cell types. In nonexpressing cells, the core promoter CpG dinucleotides were densely methylated, whereas in eNOS-expressing endothelial cells they were all unmethylated. Differential promoter methylation was further implicated in the determination of cell-specific eNOS expression by the successful induction of endogenous eNOS mRNA expression in nonexpressing cell types in response to demethylation by 5-azacytidine, a DNA methyltransferase inhibitor. It has been suggested that promoter methylation downregulates transcription by recruitment of methyl-CpG binding proteins and histone deacetylase activity, resulting in a closed and transcriptionally repressive chromatin structure.

Taking all evidence into account, a model of cell-specific control of eNOS transcription can be proposed. In this model,
upstream methylation/chromatin control regions ensure de-methylation and open chromatin conformation of the eNOS promoter in a cell-restricted manner, permitting and controlling traditional cis/trans interplay with the basal transcription machinery to achieve and direct cell-specific expression. Sp1 and Ets family members have been demonstrated to be key components of this core nucleoprotein complex.

Shear Stress
The role of disturbed blood flow in the atherosclerotic disease process is well appreciated. Therefore, the effect of changes in blood flow on eNOS expression has been an important focus of investigation.28 Animal studies emphasized the role of the endothelium in exercise- and flow-induced vasodilation and showed an upregulation of eNOS expression by exercise and shear stress.29,30 Indeed, eNOS+/− mice were found to be incapable of vascular remodeling in response to shear stress.4 At the molecular level, shear stress was found to induce eNOS mRNA expression via a transcriptional pathway.31 A 1600-bp eNOS promoter sequence was able to confer shear responsiveness to luciferase reporters.32–35 This responsiveness was lost when sequences between −1000 and −779 nt were deleted.33,35 Shear-stress responsiveness is dependent on NF-κB. Mutations in p50, a component of the NF-κB heterodimeric transcription factor, and IKKα, which regulates nucleocytoplasmic partitioning of NF-κB, markedly affect shear-stress induction of eNOS.33,35 An AP-1 element at −661 has been implicated as responsible for shear-stress-induced transcriptional activation in fetal endothelial cells.34 This suggests that regulation of eNOS during development may involve different pathways than in the adult setting.

An important issue that remains to be addressed fully is the molecular mechanism implicated in the differential effects of laminar versus disturbed flow on eNOS mRNA expression, especially in the in vivo setting. The recent development of eNOS promoter–reporter insertional transgenic mouse models will allow in vivo mechanisms to be further refined.

TGF-β
Members of the TGF-β superfamily play key roles in the regulation of growth and development. TGF-β1, in particular, is increasingly recognized as an important mediator in vascular immune injury, vessel remodeling, vasculogenesis, and angiogenesis.36,37 The recognition of type II TGF-β receptor somatic DNA mutations in atherosclerotic plaques further underlines the significance of this signaling pathway in vascular pathobiology.38 TGF-β1 has been shown to increase bovine aortic endothelial cell (BAEC) and human umbilical vein endothelial cell (HUVEC) steady-state eNOS mRNA expression in a concentration-dependent manner.39,40 The TGF-β response element was mapped to an NF-1 cis-DNA binding site at −1014 in the bovine reporter and to a region spanning −1000/−720 of the human promoter.39,40 These regions form nucleoprotein complexes that contain CCAAT transcription factor/NF-1 (CTF/NF-1).39 Formation of nucleoprotein complexes containing SMAD2 was detected on DNA probes corresponding to the TGF-β responsive region of the human promoter despite the fact that the eNOS promoter does not contain consensus SMAD2 binding sites. Depletion of SMAD2 prevented the TGF-β-induced activation of promoter reporter constructs.40 Thus, TGF-β transactivates the eNOS promoter via recruitment of ribonucleoprotein (RNP) complexes containing SMAD2 and NF-1 at distinct sites.

Cyclosporine A
Immunosuppressive therapy with cyclosporine A (CsA) has commonly been associated with hypertension. Whereas its hypertensive effects have been attributed to paracrine vasoconstrictors, CsA was also found to paradoxically augment NO production in vitro and in vivo. CsA treatment of endothelial cells in culture was shown to enhance eNOS expression through transcriptional activation.41,42 This effect of CsA on the transcriptional upregulation of eNOS was proposed to function through reactive oxygen intermediates, such as hydrogen peroxide, and reactive oxygen intermediate-induced AP-1 activity.41,42 Interestingly, hydrogen peroxide also activates eNOS transcription via a pathway dependent on Jak2, CaM kinase II, and Sp1.43,44 In vivo models of eNOS mRNA regulation are eagerly awaited.

Posttranscriptional Regulation
A surprising theme, namely the important contribution of changes in mRNA stability, emerged when studying changes in steady-state eNOS mRNA expression in models of endothelial activation. This concept has provided a new paradigm in endothelial gene regulation that is highly relevant to understanding changes in endothelial phenotype in disease. Importantly, eNOS can be a very stable mRNA species with measured half-lives that, after transcriptional arrest, average 24 to 48 hours.45–48 The high basal stability of eNOS transcripts is dependent on multiple 3′-untranslated region (3′-UTR) cis-mRNA elements that form stabilizing RNP complexes.49 After cellular activation, the mRNA half-life can decrease dramatically. If transcription rates remain unchanged, steady-state eNOS mRNA levels will decrease (Table 1).50

TNF-α and Lipopolysaccharide
TNF-α has been shown to reduce endothelium-dependent vasorelaxation in vivo and ex vivo.51,52 In the setting of atherosclerosis in which steady-state eNOS mRNA has been shown to be downregulated,9,10 the contribution of TNF-α may be crucial to the pathogenesis of the disease. Some of the earliest published studies of eNOS gene regulation identified modulation of mRNA stability as a regulatory target of pro-inflammatory cytokines.50,53 Indeed, TNF-α alone reduced the half-life of eNOS mRNA from 48 to 3 hours.54 The molecular mechanisms by which TNF-α regulates eNOS transcript stability have thus far remained elusive. TNF-α treatment of BAEC enhanced the formation of cytoplasmic RNP complexes on a 25-nt sequence of the bovine eNOS 3′-UTR. This binding activity was negatively correlated with eNOS mRNA levels in a time-dependent fashion.54 Although UV-crosslink experiments determined the molecular weight of the implicated protein to be 60 kDa, this TNF-α inducible binding factor remains unidentified.55 Three HUVEC RNP complexes form on the human eNOS 3′-UTR and are
modulated by TNF-α treatment. The protein components of these RNP complexes also remain unidentified. Formation of a 56-kDa and a 53-kDa species were upregulated on TNF-α treatment, whereas formation a 66-kDa species decreased. Adenoviral overexpression of approximately the middle third of the eNOS 3′-UTR containing mRNAs target for the smaller RNP complexes blunted the TNF-α mediated down-regulation of eNOS in infected HUVEC, implicating these complexes as destabilizing. Neither the 25-nt bovine mRNA sequence nor the human target mRNA showed overlap with the 43-nt destabilizing sequence identified in cell proliferation studies. Overall, studies of eNOS RNP complex formation suggest the interaction of multiple proteins with multiple 3′-UTR regions that may function together to regulate the stability of eNOS transcripts both basally and in response to cellular stimuli.

Recent work has demonstrated that transcription of eNOS promoter–reporter constructs is inhibited in response to TNF-α treatment, indicating that the profound downregulation of eNOS mRNA in response to TNF-α, while undoubtedly involving destabilization of eNOS mRNA, may also involve reduction of promoter activity. Lipopolysaccharide (LPS) alone, or in combination with cytokine treatment, was similarly observed to induce massive downregulation of eNOS mRNA levels. Indeed, eNOS-overexpressing transgenic mice were found to be resistant to LPS-induced hypotension and lung injury. Lu et al showed that concomitant treatment of cultured bovine coronary endothelial cells with LPS and actinomycin D decreased eNOS mRNA levels at a much faster rate compared with actinomycin D alone, implicating regulation at the level of mRNA stability. Of interest, recent work has also suggested a biphasic eNOS response to LPS. In the central nervous system, systemic treatment with LPS resulted in increased eNOS mRNA and protein in both endothelial and nonendothelial cell types. Even though a mechanism for mRNA induction is still required, it is intriguing to postulate a relationship between this transient and early upregulation of NOS activity to the “warm phase” of impending septic shock. In reality, the effects of TNF-α and LPS on eNOS expression and overall NO regulation in sepsis are expected to be much more complicated, especially in the in vivo environment.

**Entry Into Cell Cycle**

The endothelium of medium and large arterial blood vessels exhibits a very low proliferation index in vivo. In response to angiogenic, remodeling, or wounding stimuli, the endothelium undergoes marked phenotypic changes, which include changes in DNA synthesis, cell division, migration, and/or apoptosis. Although entry into the cell cycle is involved in all of these phenotypic changes, each represents a unique endothelial response with different requirements for endothelium-derived NO and eNOS expression.

Flowers et al showed that steady-state eNOS mRNA and protein expression were decreased during rapid BAEC growth. Different proliferative states were achieved by plating the cells at different dilutions. Surprisingly, nuclear run-on assays showed an increase in eNOS gene transcriptional rates in proliferating versus quiescent cells, attributing the differential eNOS mRNA expression to altered mRNA stability. Using bovine fetal pulmonary artery endothelial cells, Whitney et al showed similar steady-state eNOS mRNA and protein results using two independent in vitro models of cell proliferation. In contrast, Arnal et al sequentially followed eNOS expression in BAEC from 2 days before confluence to 6 days after confluence and noted a progressive decrease in eNOS mRNA, protein, and enzyme activity during the study period. Searles et al further pursued this in vitro model and showed that eNOS mRNA half-life was greater (<27 hours) at earlier time points and shorter (9 hours) at later time points. Transcription rates remained unchanged. Further analyses implicated a 43-nt proximal bovine eNOS 3′-UTR destabilizing sequence in the formation of an RNP complex in quiescent BAEC. Hence, in this model, the regulation of eNOS expression in different BAEC proliferative states was also attributed to alterations in eNOS mRNA stability. Remarkably, the association was reversed compared with the work of others. Whitney et al suggested that the discrepancies in results were attributable to the vascular bed of origin, because mesenteric artery endothelial cell NOS activity was not influenced by cell confluence. However, Flowers et al also studied BAEC. An in vivo rat aorta injury model and an in vitro BAEC wounding model were used by Poppa et al to study eNOS expression in endothelial regeneration and repair. Both models showed an increase in eNOS protein and enzyme activity at the proliferating wound edge. In situ hybridization performed on in vivo samples further showed an increase in steady-state eNOS mRNA expression. Clearly, it must be agreed that in vivo findings are the important ones. Mechanical forces of the circulation, paracrine factors, and cell–cell interactions can be expected to influence endothelial phenotype. This issue then becomes one of which factor or factors are the most seminal. For instance, using an in vitro wounding model, it has been suggested that wound closure was attributable mainly to the migration and not to the proliferation of endothelial cells. Endothelial migration, in turn, was found to be associated with eNOS phosphorylation and activation by the protein kinase Akt.

**Dual Regulation: Transcriptional and Posttranscriptional Effects**

**Oxidized Low-Density Lipoproteins and Lysophosphatidylcholine**

Liao et al first reported that oxidized low-density lipoproteins (ox-LDL) caused a time- and concentration-dependent decrease in steady-state eNOS mRNA and enzyme activity in human endothelial cells. This downregulation was found to occur principally at the posttranscriptional level, resulting in a reduction in eNOS mRNA half-life from 36 to 10 hours. Low concentrations of ox-LDL (≤10 µg protein/mL) may be associated with a paradoxical increase in eNOS mRNA and protein expression. Very high levels of native LDL were found to decrease eNOS mRNA expression. LPC, a major component of ox-LDL, can increase eNOS mRNA in BAEC. Perhaps this provides an explanation for the biphasic effect of varying doses of ox-LDL. LPC upregulated eNOS mRNA at the transcriptional level in a manner
dependent on the Sp1 (−104 to −94 with respect to transcription initiation) and Ets sites (−40 to −24). An important aspect of endothelial dysfunction and atherosclerosis is the reduction of NO availability and the impairment of endothelium-dependent vasorelaxation. At first glance, it is therefore surprising and contradictory that some components of ox-LDL should augment eNOS expression, even though ox-LDL predominantly decreases eNOS expression. Clearly, there are many fascinating mechanisms at play in the atherosclerotic plaque that can exert opposing effects on eNOS mRNA levels.

3-Hydroxy-3-Methylglutaryl-Coenzyme A (HMG-CoA) Reductase Inhibitors (Statins)

The mechanisms by which statins confer lipid-independent cardiovascular protection have stimulated substantial interest. Much focus has centered on the vasoactive and angiogenic properties of statins and the possible role of eNOS in mediating such effects. Simvastatin and lovastatin, for example, were shown to decrease the size of ischemic cerebral infarcts and reduce neurological morbidity through augmentation of cerebral blood flow in normocholesterolemic mice. This cerebrovascular neuroprotective effect was attributed to the upregulation of eNOS expression at the mRNA level. Treatment of eNOS+/− mice with statins did not result in protection from ischemic injury.

Statins have been shown to prevent the downregulation of eNOS mRNA by ox-LDL, hypoxia, and TNF-α. Indeed, HMG-CoA reductase inhibition has been associated with increased eNOS expression in human endothelial cells by the prolongation of its already-long mRNA half-life. Transcription arrest experiments showed a statin-induced increase in eNOS mRNA half-life from 28 hours to 46 hours and from 14 hours to 27 hours, respectively, with no major alterations in transcriptional activity. A noted prolongation of eNOS transcript half-life was consistent with the observed 3-fold increase in steady-state eNOS mRNA levels after 24 hours of statin exposure. The effects of HMG-CoA reductase inhibition on steady-state eNOS mRNA involves changes in isoprenoid synthesis and Rho GTPase protein activity. It is important to remember that posttranslational effects also figure prominently in statin effects on eNOS. HMG-CoA reductase inhibition results in the activation of protein kinase Akt and the posttranslational phosphorylation of the eNOS protein.

Vascular Endothelial Growth Factor

As the important role of vascular endothelial growth factor (VEGF) in blood vessel development, endothelial biology, and vascular disease continues to unfold, the relationship between VEGF and eNOS/NO becomes increasingly defined. For instance, using a murine ischemic limb model, Murohara et al found that VEGF failed to improve angiogenesis in eNOS+/− mice, suggesting that eNOS was a downstream mediator of VEGF-induced angiogenesis. At the molecular level, Bouloumie et al showed that VEGF increased steady-state eNOS mRNA expression in HUVEC in a time- and concentration-dependent manner. Transcription arrest studies attributed this enhanced expression to a significant increase in eNOS mRNA stability. However, the extremely short basal half-life under control conditions (undetectable in HUVEC by RT-PCR after 4 hours of actinomycin D exposure) did not correlate with the known stability of eNOS transcripts in most endothelial cells. The mechanism by which VEGF upregulates eNOS mRNA expression thus awaits confirmation, especially the transcriptional contribution of VEGF effects on steady-state eNOS mRNA and protein expression.

Hypoxia

Hypoxia has been associated with both the upregulation and downregulation of steady-state eNOS mRNA expression. Given that the body regularly deals with a wide range of oxygen tensions based on the demands of specific organs, it is not surprising that endothelial cells from various vascular beds should respond to hypoxia differently, depending on the specific in vivo and in vitro milieu, as well as the chronicity and severity of the hypoxic exposure.

Contributions from both transcriptional and posttranscriptional events were implicated as the molecular mechanism by which hypoxia induces downregulation of eNOS mRNA. In contrast to others, Arnet et al observed that hypoxia could induce upregulation of eNOS mRNA in BAEC. This was attributed to increases in transcriptional activity. Indeed, in response to the hypoxia mimic desferrioxamine, Coulet et al noted a biphasic response of eNOS steady-state mRNA in HUVEC. The initial increase in eNOS mRNA was attributed to transcriptional induction dependent on a hypoxia-dependent enhancer element at −5375 to −5366 that contains two contiguous hypoxia-
inducible factor binding sites and activates transcription via recruitment of hypoxia-inducible factor 2.

Variations in experimental design, particularly issues with oxygen equilibration in the generation of a hypoxic environment, cell type, and acuity of the model, likely account for the discrepant results observed in vitro. Also, given the evidence indicating that NO may modulate expression of the varied NOS isoform mRNAs, including eNOS itself, it remains possible that the varied in vitro and in vivo models may reflect differences in feedback regulatory mechanisms.

The histone deacetylase inhibitor trichostatin A (TSA) is known to inhibit angiogenesis in response to VEGF and hypoxia. Recently, in studies designed to elucidate the mechanism of this inhibition, Rossig et al discovered that TSA treatment of HUVEC markedly decreases steady-state levels of eNOS mRNA without affecting transcription rate as measured by nuclear runoff. TSA treatment increased eNOS promoter–reporter activity. Taken together, these data suggest that TSA modulates eNOS mRNA expression via a posttranscriptional mechanism.

**Conclusion**

eNOS has emerged in the past 10 years as an exceedingly complex and highly regulated gene with fascinating roles in many aspects of endothelial biology and pathobiology. An emerging and intriguing facet of eNOS regulation is the important contribution of both transcriptional and posttranscriptional processes to steady-state eNOS mRNA expression (Figure 1).

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**References**

transcriptional mechanisms.


Flowers MA, Marsden PA. Expression of endothelin-1 and nitric oxide is coupled to endothelial phenotype, especially growth state. Exp Nephrol. 1994;2:115–126.


Hirata K, Mik I, Kuroda Y, Sakoda T, Kawashima S, Yokoyama M. Low concentration of oxidized low-density lipoprotein and lysophosphatidylcholine upregulate constitutive nitric oxide synthase mRNA

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Flowers MA, Marsden PA. Expression of endothelin-1 and nitric oxide is coupled to endothelial phenotype, especially growth state. Exp Nephrol. 1994;2:115–126.


Hirata K, Mik I, Kuroda Y, Sakoda T, Kawashima S, Yokoyama M. Low concentration of oxidized low-density lipoprotein and lysophosphatidylcholine upregulate constitutive nitric oxide synthase mRNA

75. Vidal F, Colone C, Martinez-Gonzalez J, Badimon L. Atherogenic
concentrations of native low-density lipoproteins down-regulate nitric-
oxide-synthase mRNA and protein levels in endothelial cells. Eur
76. Zembowicz A, Tang JL, Wu KK. Transcriptional induction of endothel-
ial nitric oxide synthase type III by lysophosphatidylcholine. J Biol
77. Ceslik K, Zembowicz A, Tang JL, Wu KK. Transcriptional regulation of
endothelial nitric-oxide synthase by lysophosphatidylcholine. J Biol
78. Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA,
Liao JK. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA
reductase inhibitors mediated by endothelial nitric oxide synthase. Proc
79. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial
80. Hernandez-Perera O, Perez-Sala D, Navarro-Antolin J, Sanchez-
Pascuala R, Hernandez G, Diaz C, Lamas S. Effects of the 3-hydroxy-
3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin,
on the expression of endothelin-1 and endothelial nitric oxide synthase
81. Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric
273:24266–24271.
TF, Papapetropoulos A, Sessa WC. Regulation of endothelium-derived
nitric oxide production by the protein kinase Akt. Nature. 1999;399:
597–601.
83. Kureishi Y, Luo Z, Shiojima I, Bilak A, Fulton D, Lefer DJ, Sessa WC,
Walsh K. The HMG-CoA reductase inhibitor simvastatin activates the
protein kinase Akt and promotes angiogenesis in normocholesterolemic
84. Servos S, Zachary I, Martin JF. VEGF modulates NO production: the
85. Bouloumie A, Schini-Kerth VB, Busse R. Vascular endothelial growth
factor up-regulates nitric oxide synthase expression in endothelial cells.
86. Shen BQ, Lee DY, Zionscheck TF. Vascular endothelial growth factor
up-regulates endothelial nitric-oxide synthase expression via a KDR/FK5-1
274:33057–33063.
87. Shaull PW, Wells LB, Horning KM. Acute and prolonged hypoxia
attenuate endothelial nitric oxide production in rat pulmonary arteries by
88. Ziesche R, Petkov V, Williams J, Zakeri SM, Mosgoller W, Knoller M,
Block LH. Lipopolysaccharide and interleukin-1 augment the effects of
hypoxia and inflammation in human pulmonary arterial tissue. Proc Natl
89. Justice JM, Tanner MA, Myers PR. Endothelial cell regulation of nitric
oxide production during hypoxia in coronary microvessels and epi-
90. Resta TC, Chicone LG, Omdahl JL, Walker BR. Maintained upregu-
lation of pulmonary eNOS gene and protein expression during recovery
91. Le Cras TD, Tyler RC, Horan MP, Morris KG, Tudor RM, McMurtry
IF, Johns RA, Abman SH. Effects of chronic hypoxia and altered
hemodynamics on endothelial nitric oxide synthase expression in the
92. Phelan MW, Faller DV. Hypoxia decreases constitutive nitric oxide
synthase transcript and protein in cultured endothelial cells. J Cell
93. Arnett UA, McMillan A, Dnerman JL, Ballermann B, Lowenstein CJ.
Regulation of endothelial nitric-oxide synthase during hypoxia. J Biol
94. Coulet F, Nadaud S, Agrapart M, Soubrier F. Identification of hypoxia
response element in the human endothelial nitric oxide synthase gene
95. Kourembanas S, Marsden PA, McQuillan LP, Faller DV. Hypoxia
induces endothelin gene expression and secretion in cultured human
96. Yuhanna IS, MacRitchie AN, Lantin-Hermoso RL, Wells LB, Shaull
PW. Nitric oxide (NO) upregulates NO synthase expression in fetal
intrapulmonary artery endothelial cells. Am J Respir Cell Mol Biol.
97. Chen JX, Berry LC, Tanner M, Chang M, Myers RP, Meyrick B. Nitric
oxide donors regulate nitric oxide synthase in bovine pulmonary artery
HS, Lee SK, Chung HY, Kim CW, Kim KW. Histone deacetylases
induce angiogenesis by negative regulation of tumor suppressor genes.
99. Deroanne CF, Bonjean K, Servotte S, Devy L, Colige A, Clusset N,
Blacher S, Verdin E, Fouldart JM, Nusgens BV, Castronovo V. Histone
deacetylases inhibitors as anti-angiogenic agents altering vascular endo-
100. Rossig L, Li H, Fisslthaler B, Urbich C, Fleming I, Forstermann U,
Zeher AM, Dimmeler S. Inhibitors of histone deacetylation down-
regulate the expression of endothelial nitric oxide synthase and com-
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