Nitric oxide (NO) plays an important role not only in physiological conditions, such as vasodilation, inhibition of platelet aggregation, and regulation of gene transcription, but also in atherosclerosis development. NO is synthesized from L-arginine by a family of 3 NO synthases (NOS): neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). eNOS possesses an N-terminal oxygenase domain containing single heme and tetrahydrobioperin (BH-4)-binding sites, a C-terminal reductase domain containing single binding sites for flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and NADPH, and a central calmodulin (CaM) binding site. eNOS is specifically and constitutively expressed in endothelial cells normally localized in caveolae, endoplasmic reticulum, and nuclear envelope. In caveolae, eNOS associates with growth factor or hormone receptors. On ligand-receptor binding, the receptor-associated eNOS will be phosphorylated, homodimerized, and coupled with CaM together with cofactors BH4, heme, FAD, FMN, and NADPH to form a complex. The complex will be translocated to endoplasmic reticulum via caveolin and oxidize L-arginine to release NO. However, under oxidative stress conditions, caused by atherosclerotic risk factors—such as cholesterol overloading, oxidized LDL, smoking, diabetes mellitus, etc—eNOS will be uncoupled to produce superoxide. Thus, eNOS can produce both NO and superoxide, exerting atheroprotective and proatherogenic effects, by which it modulates gene transcription. Recent reports have shown that activated eNOS can translocate into nucleus where it regulates gene transcription. However, the underlying mechanism remains unclear.

ERα and eNOS Form a Complex That Enhances Telomerase Activation

Estrogen is an important atheroprotective molecule, possessing multiple biological effects on vasculature. There are two estrogen receptor (ER) isoforms, ER alpha (ERα) and ER beta (ERβ), residing in caveolae associated with eNOS. On ligand binding, activated ERα and ERβ translocate into nucleus and bind to the estrogen response element (ERE) in the promoter region of target genes. Estrogen modulates eNOS activity through transcriptional regulation and phosphorylation of eNOS protein. There are ERE binding sites in the eNOS gene promoter. Estrogen binding to ERα can also result in activation of intracellular kinases, eg, Akt that phosphorylates eNOS leading to NO release. Several reports have shown that eNOS activation and NO production modulate telomerase activity, which plays a pivotal role in the determination of the lifespan of a cell. However, a report by Hong et al contradicts this evidence. Interestingly, Grasselli et al reported a striking find in this issue of Circulation Research, that ERα and eNOS formed a complex in the ERE sites in the promoter region of telomerase catalytic subunit gene (hTERT) leading to the increase of hTERT transcription and telomerase activity. This finding creates a new perspective that eNOS may function as a coactivator in the regulation of gene transcription.

They found that estrogen 17-β-estradiol (E2) induced hTERT gene expression, which was blocked by the addition of NOS inhibitor 7-nitroindazole, indicating the involvement of eNOS. Direct evidence came from the experiments of the reconstitution of eNOS in pulmonary endothelial cells derived from eNOS knockout mouse. Transfection of a constitutively active eNOS mutant S1177D or treatment with NO donor (DETA-NO) increased hTERT transcription. This effect required an intact ERE binding site in the DNA sequences, indicating the essential role of ERα as a transcription factor. The colocalization of ERα and eNOS in the nucleus, especially in the hTERT promoter region, indicates that ERα and eNOS form a complex in the transcription machinery, and that eNOS just functions as a coactivator. Both ERα and eNOS reside in the caveolae. It is possible that estradiol-bound ERα and eNOS form complex in the caveolae and translocate into nucleus together in a complex form. It is then recruited to the ERE in hTERT gene promoter. The key element of this elegant study provides us with a new concept, that membrane resident molecules can directly transduce extracellular signal into nuclear gene transcriptional regulation, through interaction with growth factor or hormone receptors functioning as coactivators (Figure). It also reminds us to reevaluate whether eNOS functions as coactivator in other signal transduction pathways, such as VEGF–A–mediated gene regulation, in which eNOS is co-translocated into nucleus with receptor Flk-1/KDR.

Potential Impact of ERα and eNOS Complex

Despite these carefully performed studies, a number of questions regarding the exact function of eNOS as a coactivator remain unanswered. What types of modifications, if any, occur in the nuclearized, especially DNA-bound, eNOS? There are several posttranslational modifications such as acylation, phosphorylation, and S-nitrosylation at different sites of eNOS protein, that affect its cellular compartment location and function. Klinz et al reported that phosphorylated eNOS Ser114 localized in nucleus, producing superoxide...
anions during cell mitosis in human mesenchymal stem cells. In this study, mutant eNOS S1177D (which mimics phosphorylation at this site) restored the eNOS activity in eNOS knockout endothelial cells, and increased hTERT transcription. However, whether the nuclearized ERα-bound eNOS is phosphorylated at Ser1177 is unclear. Thus, the modifications of nuclearized eNOS remain to be determined. In addition, does the ERα–eNOS complex produce NO or superoxide anions locally? As described above, eNOS can produce both NO and superoxide depending on whether coupling or uncoupling to cofactor BH4.1–8 To produce NO, eNOS needs dimerization and recruitment of cofactors, including CaM, heme, BH4, FAD, FMN, and NADPH, whereas undimerized and uncoupled eNOS can produce superoxide. Goetz et al19 reported that estradiol-induced eNOS nuclearization is calcium dependent, and that NO donor could increase hTERT transcription, suggesting that nuclearized eNOS produces NO locally. However, whether CaM remains in the ERα–eNOS complex is unclear. Meanwhile, NO can react with superoxide anion, producing peroxyxinitrite20 and increasing the production of superoxide by eNOS and iNOS. Thus, further experiments are needed to determine whether ERα and eNOS form heterodimer, consisting of one molecule of each or tetramer, and whether cofactors are required in the ERα–eNOS complex.

Whatever is produced by the ERα–eNOS complex, ie, either NO or superoxide, these active molecules could modify eNOS-associated proteins and DNA locally. Therefore, the modification of ERα, other transcription factors, and coactivators that are recruited to the complex, local DNA sequence and histones need to be determined. Local NO or superoxide production may also modify corepressors to exclude these factors from the complex. Furthermore, we can question whether eNOS directly modifies the arginine residues in the associated transcription factors, like ERα and local histones. Do these arginine residues function as electron recipients like L-arginine in the production of NO by eNOS? The direct electron transferring from the reductase unit of eNOS to the arginine residues of the associated proteins will modify the arginine residues and cause the conformational change of the proteins. The answers to these questions will enhance our knowledge concerning eNOS functions, especially as coactivator, and provide some new strategies to intervene vascular disease through eNOS.

In summary, ERα–eNOS interaction in caveolae plays a crucial role in vascular homeostasis. The report by Grasselli et al17 provided new evidence that activated ERα–eNOS complex translocates into nucleus forming heterodimer or tetramer on the ERE binding sites in the promoter of hTERT gene. This results in increases in hTERT gene transcription that regulates telomerase activity (Figure). Further studies on the mechanisms of gene transcription or epigenetic modification induced by ERα–eNOS complex, will lead to new findings of some targets for therapeutic intervention for vascular diseases.

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References


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