Histol Histopathol (2004) 19: 585-605

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

To NO or not to NO: 'where?' is the question

R. Govers[1] and S. Oess[2]

[1]Department of Vascular Medicine, UMC Utrecht, Utrecht, the Netherlands,

[2]Institute for Biochemistry II, Johann Wolfgang Goethe University, Frankfurt am Main, Germany

Summary. Nitric oxide (NO) is a gaseous radical with unique biological functions essential for the cardiovascular system, host defense and neurotransmission. For two decades it was thought that NO was able to diffuse freely across relatively long distances and to traverse major parts of the cell, if not multiple cell layers. However, NO has been proven to be extremely reactive: it reacts with other reactive oxygen species, heavy metals, as well as with cysteine and tyrosine residues in proteins. In accordance, it is now widely accepted that once NO is generated, it is very short-lived and diffuses only over a short distance. This urges for the local production of NO and the localization of NO synthases in the proximity of their downstream targets. This review discusses the highly organized localization of NO synthases, with the endothelial isoform (eNOS) as its main focus, since from this synthase most is known about its subcellular localization and regulation.

Key words: Nitric oxide, Nitric oxide synthase, eNOS, Intracellular localization, Review

Implications of high NO reactivity

NO, elected "Molecule of the Year" by Science in 1992, is a diffusible second messenger that has a half-life of a few milliseconds *in vivo*. This is caused by its ability to react with various substances in an extremely rapid fashion, which is mainly due to its unpaired electron (Hanafy et al., 2001). One of its main physiological actions involves its reaction with the heme-contained iron within the enzyme guanylate cyclase in vascular smooth muscle cells, resulting in dilation of the bloodvessel (Gruetter et al., 1980). Other transition-metal-containing proteins that undergo iron nitrosylation include the bacterial transcription regulator Fur (D'Autreaux et al., 2002) and catalase (Brown, 1995). Intriguingly, NO can also react with the iron in the heme of NO synthase (NOS) molecules, resulting in inhibition of NOS dimer formation (Chen et al., 2002).

In addition to NO-mediated regulation of enzymes via its reaction with transition metals, at high concentrations NO can also modify protein function by direct interaction with two amino acid residues.

First, NO may react with free thiol groups of cysteine residues (a process called S-nitrosylation or nitrosation), which leads to the formation of more stable nitrosothiols, with half-lives up to several minutes (Jaffrey et al., 2001). Nitrosothiol formation can be the result of a direct reaction with NO or of an oxidative nitrosylation reaction involving the preformation of peroxynitrite (Espey et al., 2002). Examples are S-nitrosoglutathione and S-nitroso-cystein-glycine which have important regulatory functions in synaptic neurotransmission in the brain (Salt et al., 2000). Other S-nitrosylated proteins include the NMDA and ryanodine receptors (Xu et al., 1998; Jaffrey et al., 2001), ras (Lander et al., 1997), caspases (Mannick et al., 2001), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Molina y Vedia et al., 1992) and DNA repair proteins (Laval and Wink, 1994). Recently, using an adapted version of the yeast two-hybrid system, it was shown that nitrosylation events play a role in the regulation of several protein-protein interactions, further delineating the importance of this modification (Matsumoto et al., 2003). Importantly, S-nitrosylation has been indicated not to occur in an at random fashion, but to be dependent on the presence of specific consensus motifs (Hess et al., 2001). How such motifs can determine S-nitrosylation events is currently unknown. Possibly, these motifs influence the accessibility of the thiol groups to NO.

Second, NO can react with the phenol moiety in tyrosine residues, a process also known as tyrosine nitration (Hanafy et al., 2001). This process requires the preformation of peroxynitrite and usually has detrimental effects on protein function. Proteins that can be nitrated on tyrosine residues *in vivo* include actin (Aslan et al., 2003), histone proteins (Haqqani et al., 2002), protein kinase C (Knapp et al., 2001), prostacyclin synthase (Zou et al., 1998), and

Offprint requests to: Roland Govers, Department of Functional Genomics, Center for Neurogenomics and Cognitive Research (CNCR), Free University (VU) and VU Medical Center (VUmc), WN building, A4 wing, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands. Fax: +31-20-4446926. e-mail: roland@cncr.vu.nl

transcription factor STAT1 (Llovera et al., 2001). Tyrosine nitration predominantly occurs in case of inducible NOS (iNOS), since this NOS isoform is capable of producing rather large quantities of NO (Nishida and de Montellano, 2001). This is conform to the physiological relevance of iNOS: destruction of tumor cells, bacteria and other microbes invading the body (Nathan, 1997).

Another important chemical reaction is the reaction of NO with the radical gas superoxide (O_2^-), leading to the formation of yet another oxidative substance: peroxynitrite ($ONOO^-$). As discussed above, the formation of peroxynitrite may result in S-nitrosylation and tyrosine nitration of proteins with a concomitant change in their function, e.g. peroxynitrite decreases NO generation by endothelial NOS (eNOS) via inhibition of Akt (Zou et al., 2002). In addition, peroxynitrite can also react with NOS cofactor tetrahydrobiopterin (BH4), thereby directly decreasing NOS activity (Kuzkaya et al., 2003). Interestingly, superoxide can be generated by NO synthases themselves, in case of so-called "NOS uncoupling", i.e. uncoupling of NADPH oxidation. In case of NOS uncoupling, NOS-generated superoxide may even react with NO produced by another NOS molecule, possibly within the same NOS dimer, thereby even further reducing the availability of NO. NOS uncoupling has been demonstrated for all three NOS isoforms in vitro and is dependent on the intracellular levels of their substrate arginine and cofactor tetrahydrobiopterin (BH4) (Vasquezvivar et al., 1998; Pou et al., 1999). For eNOS, uncoupling has also been reported to occur *in vivo* (Stroes et al., 1997; Guzik et al., 2002; Shi et al., 2002a) and is at least in part determined by hsp90 action and plasma LDL levels (Pritchard et al., 2002; Shi et al., 2002b).

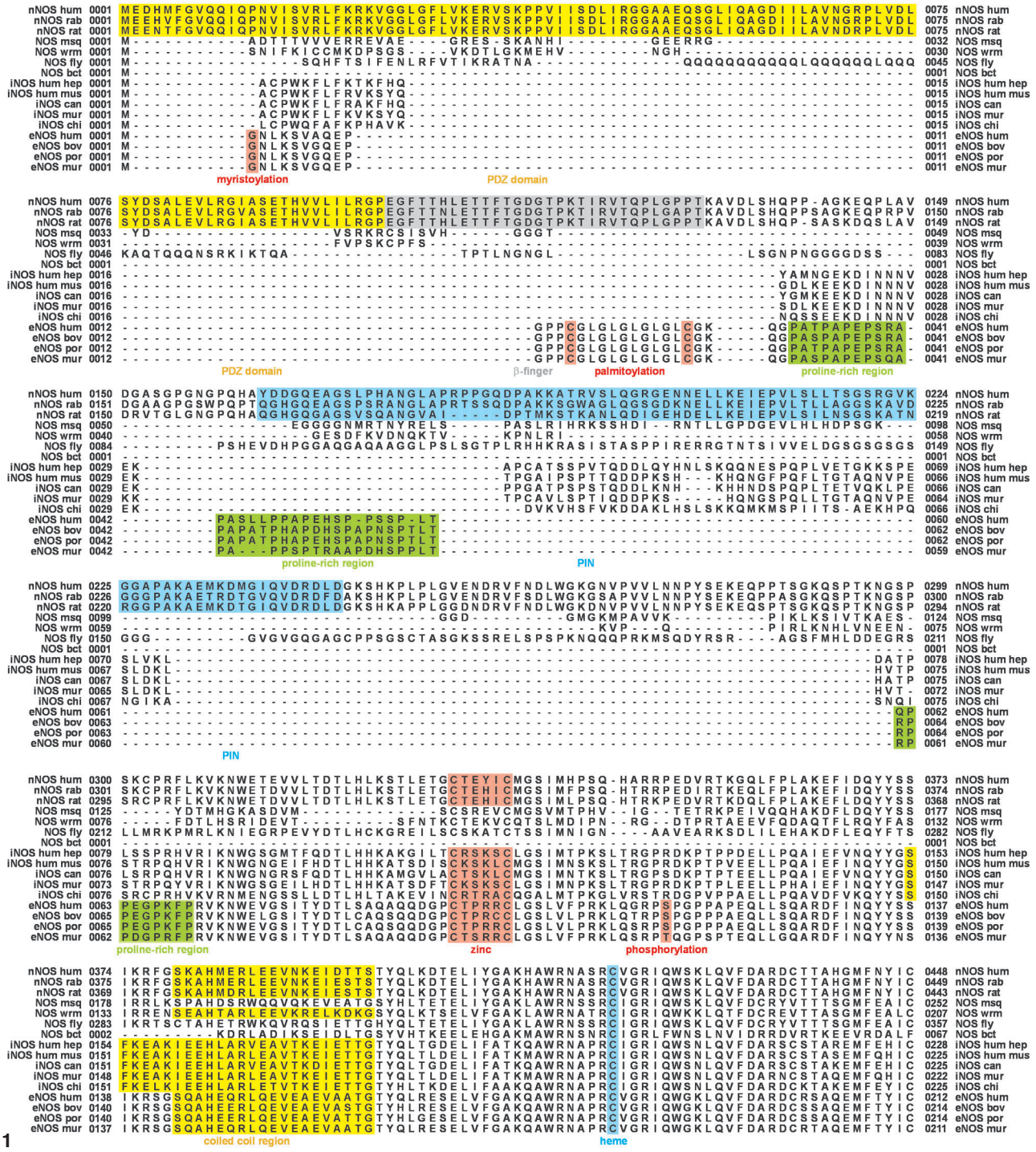
The limited half-life of NO due to its high reactivity is compensated for by two mechanisms. First, NO can react with molecules that are able to release NO at a later stadium, thereby buffering NO. For instance, in blood the reaction of NO with hemoglobin, resulting in the formation of S-nitrosohemoglobin, permits the transport of NO to tissues in which NO may be released together with oxygen via transnitrosation of a transport protein. Another S-nitrosylated protein in the circulation is albumin, which is considered to be the main NO carrier in serum (Stamler et al., 1992). Within tissues, NO 'stores' involve S-nitrosated proteins from which NO may be transferred to target proteins via low molecular weight thiols as well as nitrite (Rodriguez et al., 2003). Even though diffusion of NO through membranes is considered to be more effective than its diffusion through cytosol, the relevance of this process *in vivo* may be limited. A recent study indicated the targeted delivery of NO from one cell to another that involves the formation of NO-membrane protein transport complexes, rather than the diffusion of NO across membranes (Pawloski et al., 2001).

Second, the short reactive life of NO urges for its defined local synthesis, especially when low quantities of NO are generated, which is the case for eNOS and neuronal NOS (nNOS). This implicates the need for the juxtaposition of eNOS and nNOS with their targets at specific localizations (or microdomains) within cells. For eNOS, its intracellular localization has been studied for more than a decade. Several research groups discovered that within endothelial cells, eNOS is present at the plasma membrane where it can be found at caveolae (Shaul et al., 1996). In addition, eNOS is also present at the Golgi complex and in the cytosol (Robinson et al., 1995; Sessa et al., 1995). Recently, cell-cell contacts have been described as important sites where eNOS is specifically enriched (Govers et al., 2002a). Also for nNOS and iNOS it has become evident that, though both enzymes are not anchored in the membrane bilayer, they are enriched at particular parts of the cell. The cellular localization of NO synthases is governed by isoform-specific sequences in their primary structures (Fig. 1). The relevance of the presence of NO synthases at specific cellular compartments is discussed in this review. Related to the specialized localization of the NOS enzymes, also the NO targets are restricted in their localization and are present in close proximity of NOS. A clear example is eNOS main effector guanylyl cyclase. This enzyme is partially associated with the plasma membrane in heart and platelets, which highly sensitizes it for NO, further indicating a highly spatially confined regulation of NO signaling (Zabel et al., 2002).

eNOS

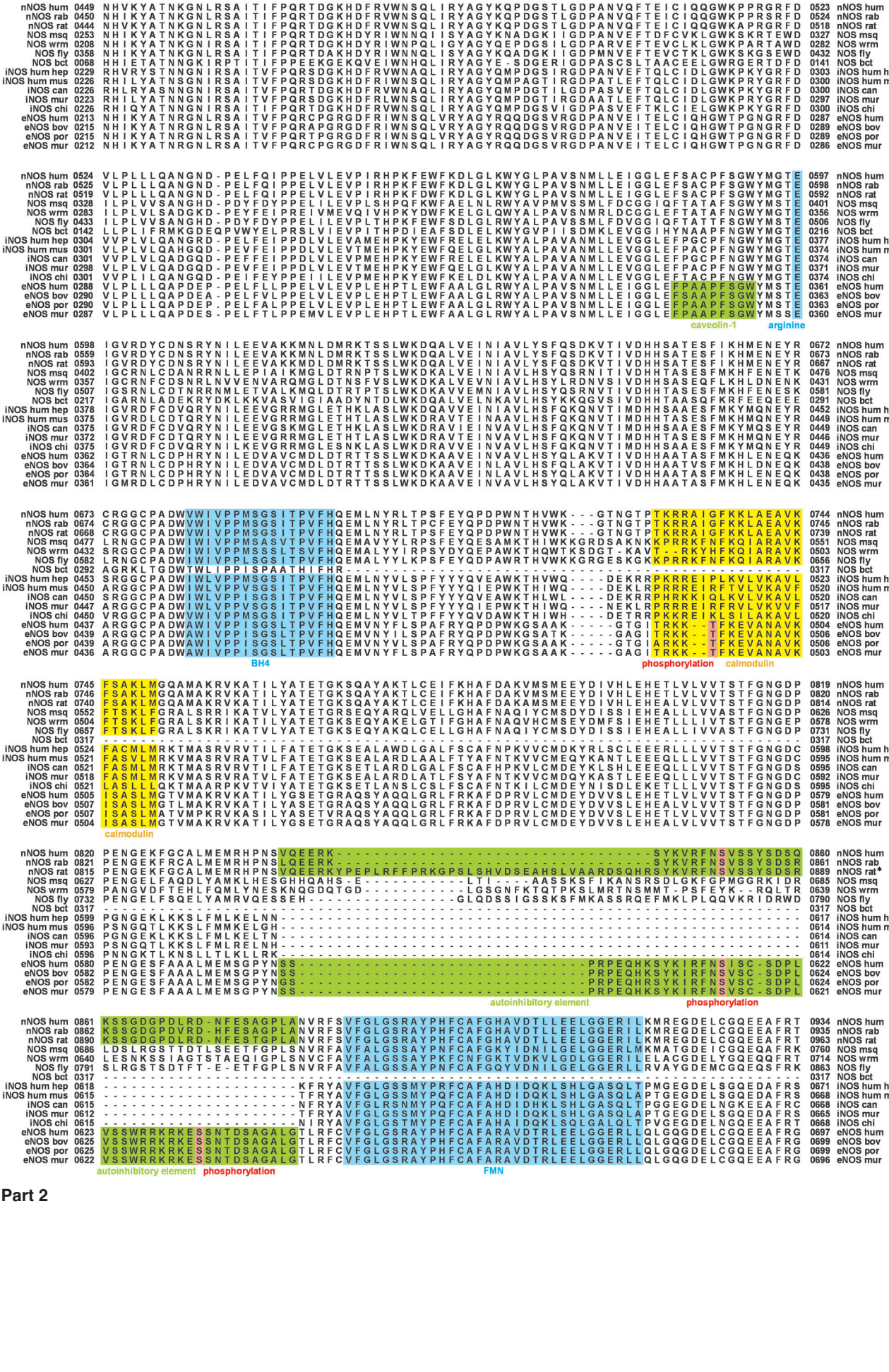
eNOS is mainly expressed in endothelium, cardiomyocytes and blood platelets, where it contributes for a large part to the role of the endothelium as the first line defense mechanism against the development of vascular disease. NO generated by eNOS has a wide range of functions: it induces vasodilation, and inhibits platelet aggregation, adhesion of platelets to the endothelium, expression of adhesion molecules, and migration and proliferation of smooth muscle cells. Furthermore, eNOS-generated NO is important for angiogenesis, and has a major role in the regulation of the permeability of the endothelium (Gewaltig and Kojda, 2002). In accordance, eNOS gene therapy has been shown to inhibit neointima formation after balloon angioplasty (Chen et al., 1998), to inhibit intimal hyperplasia of vein grafts (Ohta et al., 2002) and to improve relaxation of atherosclerotic vessels (Ooboshi et al., 1998), while eNOS hyperactivity in caveolin-1 knock-out mice results in hyperpermeability of the microvasculature (Schubert et al., 2002).

Besides the regulation of eNOS at the level of expression by factors such as shear stress (Noris et al., 1995), hypoxia (McQuillan et al., 1994), TNF-α (Alonso et al., 1997) and VEGF (Bouloumie et al., 1999), eNOS activity is coordinated by a complex combination of substrate/cofactor availability, protein-protein interactions, phosphorylation, acylation and cellular localization. Even while a lot is known about the

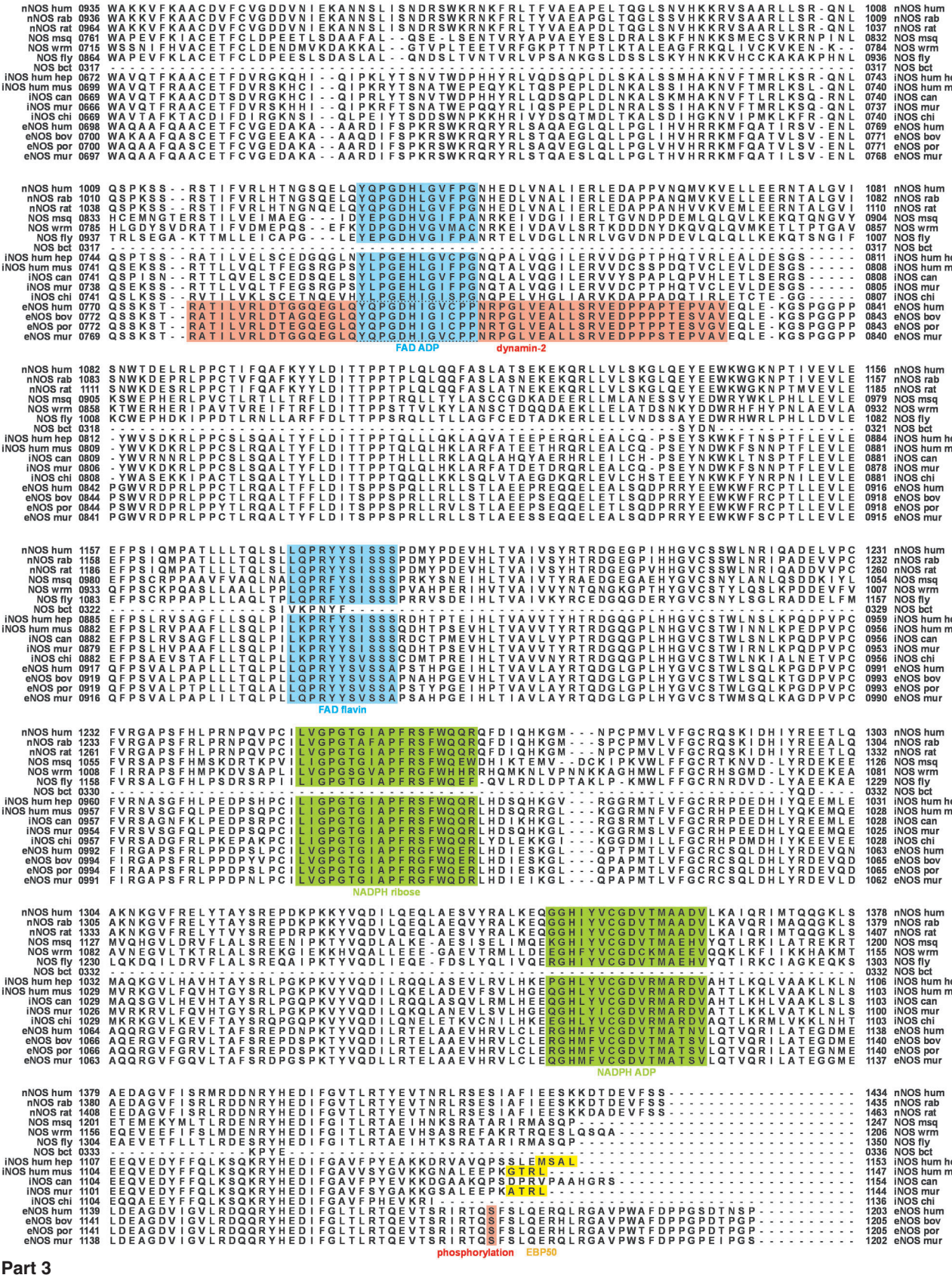


Part 1

Fig. 1. Primary structure of NO synthases. nNOS, iNOS, and eNOS consist of an N-terminal oxygenase domain and a C-terminal reductase domain, linked by a calmodulin binding motif. The NOS oxygenase domain shares homology with the NOS homologue from bacteria as shown. The NOS reductase domain resembles mammalian cytochrome P450 reductase (sequence not shown) with an overall 30% sequence homology. The synthesis of NO and co-product L-citrulline requires NOS substrates L-arginine, oxygen and NADPH, and cofactors tetrahydrobiopterin (BH4), flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN). Furthermore, NOS contains binding sites for heme, calmodulin and zinc. Residues that are acylated, phosphorylated or involved in binding of other proteins or NOS cofactors are indicated. Coiled coil regions were predicted according to the method of Lupas (Lupas et al., 1991) (http://www.ch.embnet.org/software/COILS_form.html). Proline-rich regions were identified using a protein motif identifier engine and the Hits database (Pagni et al., 2001) (http://hits.isb-sib.ch/cgi-bin/PFSCAN). Protein accession numbers: human nNOS, P29475; rabbit nNOS, O19132; rat nNOS, NP_434686; mosquito *Anopheles stephensi* NOS, O61608; tobacco hornworm NOS, AAC61262; *Drosophila melanogaster* NOS, T13254; *Bacillus subtilis* NOS, NP_388644.1; human hepatocyte iNOS, P35228; human muscle iNOS, AAC83554; canine iNOS, AAC78630; murine iNOS, P29477; chicken iNOS, Q90703; human eNOS, P29473; porcine eNOS, Q28969; murine eNOS, P70313. *:rat nNOS residues 840-873 are absent in rat nNOS isoform P29476 (Magee et al., 1996).


caveolin-1
arginine
BH4
phosphorylation
calmodulin
calmodulin
autoinhibitory element
phosphorylation
autoinhibitory element
phosphorylation
FMN

Part 2


FAD ADP
dynamin-2
FAD flavin
NADPH ribose
NADPH ADP
phosphorylation
EBP50

Part 3

localization and interacting partners of eNOS, the complex molecular mechanisms regulating eNOS localization and activity are remarkably poorly understood. The results of many years of eNOS research starting with its cloning more than a decade ago are discussed in the following sections.

eNOS regulation by signal transduction pathways

The signal transduction pathways that are involved in eNOS activation are quite well understood. The factor which is the prime modulator of NOS function is calmodulin (CaM) (Bredt and Snyder, 1990). Upon agonist-induced rises in intracellular calcium levels, Ca^{2+}-bound CaM interacts with nNOS and eNOS to initiate their enzymatic activity. With a tightly bound CaM that is constitutively associated, iNOS on the other hand is fully active under basal calcium concentrations (Abu-Soud and Stuehr, 1993). The binding of calmodulin induces the activation of the NOS reductase domain and the shuttling of electrons from the reductase domain to the oxygenase domain (Craig et al., 2002). Agonists that induce eNOS activation by increasing cytosolic Ca^{2+} levels include acetylcholine, bradykinin, serotonin, and histamine.

Shear stress-induced eNOS activation is less sensitive to Ca^{2+} fluxes. Fluid shear stress causes an increase in PI 3-kinase activity and the activation of serine/threonine kinases Akt (also known as protein kinase B or PKB) (Dimmeler et al., 1999) and protein kinase A (PKA) (Boo et al., 2002), with the latter most probably mediating the shear-stress-induced phosphorylation of (human) eNOS at Ser-1177 (Boo et al., 2002). This phosphorylation increases the enzymatic activity in relatively low intracellular calcium levels, though chelation of calcium still abolishes eNOS activity, indicating that phosphorylation at Ser-1177 sensitizes eNOS for Ca^{2+}-calmodulin binding (Caulin-Glaser et al., 1997; Dimmeler et al., 1999).

VEGF-stimulated Akt kinase also phosphorylates eNOS at residue Ser-1177, with a concomitant increase in eNOS activity (Fulton et al., 1999). In addition, estradiol and bradykinin activate eNOS in an Akt-dependent manner as well (Haynes et al., 2000; Harris et al., 2001b), though bradykinin-induced increases in Ser-1177 phosphorylation and activation of eNOS require activated PKA as well (Bae et al., 2003). Hsp90, which interacts directly with eNOS, may play a regulatory role in the process of Akt-mediated eNOS activation, by acting as a molecular scaffold for the two proteins (Russell et al., 2000; Fontana et al., 2002). Recently the c-Src kinase was found to be activated by estradiol upstream of PI 3-kinase and Akt via direct interaction with the estrogen receptor (Haynes et al., 2003), while protein kinase C has been implicated in VEGF-induced eNOS activation (Kou et al., 2002). Moreover, the VEGF receptor can be used by factors other than VEGF to induce eNOS activation, i.e. via transactivation. This has been reported for the platelet-derived bioactive lipid sphingosine 1-phosphate which is able to induce tyrosine phosphorylation of the VEGF receptor (Tanimoto et al., 2002).

In addition to Ser-1177, other phosphorylation sites have been identified in eNOS (Fig. 1). Phosphorylation at Ser-114 and Thr-495 (human eNOS) has a negative impact on eNOS activity (Harris et al., 2001b; Kou et al., 2002), the latter by inhibiting the binding of calmodulin (Fleming et al., 2001), while phosphorylation at Ser-615 and Ser-633 increases Ca^{2+}-calmodulin sensitivity and its overall activity, respectively (Michell et al., 2002). Ser-615 and Ser-633 are located within the auto-inhibitory element of eNOS and nNOS (Fig. 1), suggesting that phosphorylation of these residues may impede a structural change in the auto-inhibitory loop, thereby enhancing the enzymatic activity of the synthase. Dephosphorylation of eNOS has been reported to be mediated by the protein phosphatases calcineurin and PP2A (Harris et al., 2001b; Greif et al., 2002; Kou et al., 2002). Taken together, these processes urge for a coordinated mechanism of eNOS activation involving both phosphorylation and dephosphorylation steps.

eNOS intracellular localization

An important characteristic of the intracellular localization of eNOS is its dependence on the origin of the endothelial cell. For instance, endocardial and coronary arterial endothelium express more eNOS than cardiac venous and capillary endothelium, while in the endocardial endothelium, relatively more eNOS is present at peripheral cell borders compared to arterial and venous endothelium (Andries et al., 1998). This is inherent to the fact that different vessel types have different specific functions (Cines et al., 1998). Also, endothelial cells from different vessels have different amounts of caveolae, which most probably has its effect on eNOS function. In addition, the amount of caveolae tends to decrease once endothelial cells are being cultured. All these issues have a negative impact on the progress in eNOS research, in particular since different research groups use endothelial cells from different origins for their experiments (i.e. similar vessels from different tissues or even functionally and morphologically different vessel types).

eNOS at caveolae

One particular characteristic of all endothelial cells is the high abundance of caveolae. Endothelial caveolae contain eNOS and have been the main focus of eNOS localization studies (Shaul et al., 1996) (Fig. 2). Caveolae distinguish themselves from the rest of the plasma membrane by their typical morphology (flask shape) and by their specific lipid composition: they have a high amount of glycosphingolipids and cholesterol. The most abundant as well as best studied protein that is present at caveolae is the 22 kDa protein caveolin-1. Caveolin-1 interacts with a multitude of proteins that are

located at the cytoplasmic surface of caveolae. Its so-called scaffolding domain, a hydrophobic domain between the amino-terminus and the putative membrane domain, is involved in membrane binding as well as in the interaction of caveolin-1 with heterotrimeric G protein subunits, Src tyrosine kinases, H-Ras, EGF receptor, protein kinase C, and other caveolin-1 molecules (Li et al., 1996; Couet et al., 1997; Oka et al., 1997; Schlegel et al., 1999). By its ability to interact with so many signaling molecules and to suppress their basal activity, caveolin-1 recruits these proteins into a complex signaling network at the site of caveolae, thus enabling them to regulate specific signal transduction pathways in a highly coordinated fashion once their activation is initiated.

Importantly, eNOS also interacts with the caveolin-1 scaffolding domain. This interaction renders eNOS inactive and requires eNOS residues ^{350}FSAAP FSGW^{358}, which are well conserved within the other NOS isoforms. Mutation of this domain abolishes the ability of caveolin-1 to inhibit eNOS activity and peptides derived from the scaffolding domain of caveolin-1 and -3 (but not the analogous region of caveolin-2) inhibit eNOS, nNOS and iNOS *in vitro* (Garcia-Cardena et al., 1997). Interestingly, systemic injection of a cell permeable peptide resembling the caveolin-1 scaffolding domain in mice reduces eNOS activity (Bucci et al., 2000), supporting the notion that the interaction with caveolin-1 is an important mechanism for the control of eNOS activity.

The localization of eNOS at caveolae is not mediated by its interaction with caveolin-1 but by myristoylation and palmitoylation at its amino-terminus (Shaul et al., 1996). Studies by the research group of

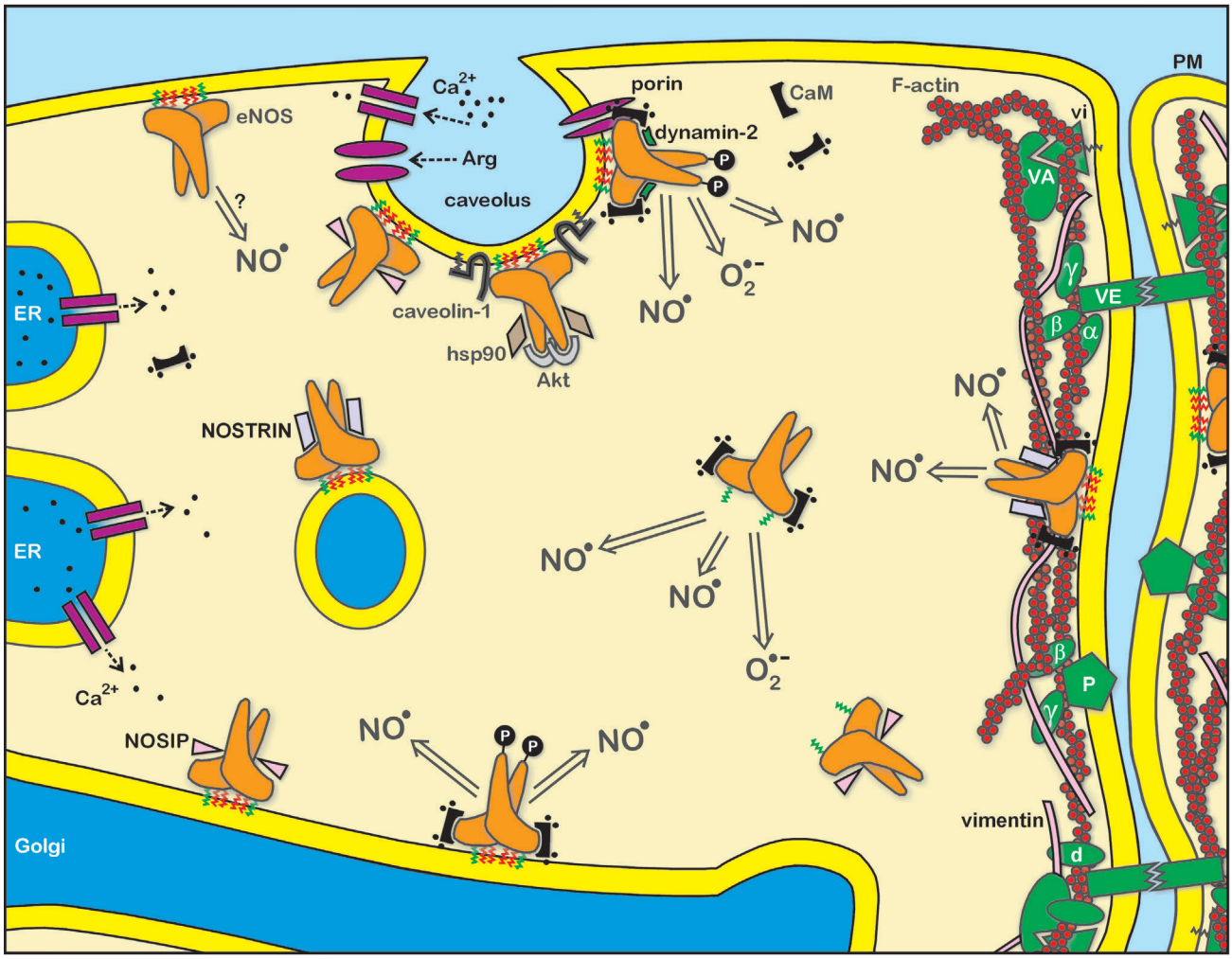


Fig. 2. Model for localization and regulation of eNOS in the endothelial cell. For details see text. Green-colored proteins represent structural proteins of the cell-cell contacts: α, α-catenin; ß, ß-catenin; γ, γ-catenin (plakoglobin); VE, VE-cadherin; P, PECAM-1; vi, vinculin; VA, VASP; d, desmoplakin; PM, plasma membrane.

Sessa have revealed that co-translational myristoylation at Gly-2 renders eNOS membrane-bound while subsequential post-translational palmitoylation at Ser-15 and Ser-26 targets eNOS to caveolae (Sessa et al., 1995; Garcia-Cardena et al., 1996). While eNOS myristoylation is irreversible, palmitoylation on the other hand is a reversible process. Importantly, palmitoylation-deficient eNOS is localized to the Golgi area only and is less active than wild-type enzyme. However, *in vitro* this mutant is as active as wild-type eNOS indicating that caveolar localization of eNOS is required for its optimal activity and that complex formation with caveolin-1 keeps eNOS in an inactive yet activatable state (Liu et al., 1996). After stimulus-induced increases in intracellular calcium levels, calcium-bound calmodulin displaces caveolin-1 from eNOS, resulting in NO production by eNOS and in a redistribution of eNOS from caveolae (Michel et al., 1997).

Unfortunately, at present it is unknown how much of the plasma membrane pool of eNOS is localized at caveolae. Recent studies, demonstrating that only 5% of membrane-associated eNOS is bound to caveolin-1, suggest that the regulatory role of caveolin-1 (and caveolae) in modulating eNOS activity may be very limited (Pritchard et al., 2002). Nevertheless, mice lacking caveolin-1 have no endothelial caveolae and clearly show an enhanced eNOS activity, suggesting an important role for caveolin-1 and caveolae in eNOS regulation (Drab et al., 2001). In addition, activation of eNOS at caveolae is facilitated by the local enrichment of factors that are crucial for its activity. Caveolae harbour the arginine channel CAT-1 (McDonald et al., 1997), calcium channels (Fujimoto, 1993), enzymes that mediate regeneration of arginine (Flam et al., 2001), and receptors for eNOS agonists (Haasemann et al., 1998; Chambliss et al., 2000).

Other caveolar proteins that are known to interact with eNOS include hsp90 and dynamin-2. At least in part, the eNOS-hsp90 interaction takes place at caveolae in a complex that also contains caveolin-1 (Gratton et al., 2000). Moreover, hsp90 binds eNOS in close proximity of the caveolin-1 binding motif (Fontana et al., 2002). Several functions have been attributed to hsp90-eNOS binding: hsp90 may increase the affinity of Ca^{2+}-calmodulin for eNOS (Song et al., 2001; Takahashi and Mendelsohn, 2003), hsp90 may be regulating eNOS uncoupling (Pritchard et al., 2001; Song et al., 2002), and/or hsp90 may act as a scaffold for eNOS and Akt (Fontana et al., 2002; Takahashi and Mendelsohn, 2003). Recently, hsp90 was shown to function as a scaffold for eNOS and its downstream target soluble guanylate cyclase (Venema et al., 2003).

Recent findings have indicated both the interaction as well as colocalization of eNOS and dynamin-2 (Cao et al., 2001). A dynamin-2 proline-rich region interacts with part of the eNOS FAD binding domain, probably via an SH3-like domain (Cao et al., 2003). This interaction potentiates eNOS activity *in vitro* as well as *in vivo*, most likely by increasing the electron transfer between FAD and FMN. Since both dynamin-2 and eNOS are present at caveolae as well as the Golgi complex, it is not completely clear at present where dynamin-2 has its effect on eNOS. The interaction of dynamin-2 with eNOS is enhanced after calcium ionophore stimulation, raising the possibility that dynamin-2 may bind eNOS upon Ca^{2+}-calmodulin-induced dissociation of the eNOS-caveolin-1 complex (Cao et al., 2001). Possibly, caveolin-1 interferes with dynamin-2 binding.

Another binding partner which has been suggested to interact with eNOS in caveolae is the voltage-dependent anion channel porin (Sun and Liao, 2002). The localization of porin in caveolae may be defined by its ability to bind cholesterol. The eNOS-porin interaction is enhanced by calcium mobilizing agonists, suggesting a potential role of intracellular calcium in regulating this interaction. Importantly, the interaction augments eNOS activity.

While eNOS interacts with caveolin-1 in endothelial cells, it binds to caveolin-3 in cardiomyocytes (Feron et al., 1998a). This interaction is also inhibitory. In cardiomyocytes, eNOS is targeted to caveolae that also harbour L-type calcium channels as well as acetylcholine and ß-adrenergic receptors, all involved in eNOS activation (Feron et al., 1997; Darby et al., 2000; Rybin et al., 2000). NO that is produced here inhibits the L-type calcium channels, suggesting an inhibitory feedback regulation (Barouch et al., 2002).

In summary, the localization of eNOS at caveolae in endothelial cells and cardiomyocytes is clearly important for its enzymatic activity. In addition, considering the effect of NO on protein function (see section 1), it cannot be excluded that caveolar localization of eNOS is important for caveolae function. Hence, it is tempting to speculate that caveolar eNOS may have a role in regulating signal transduction pathways that emerge from caveolae.

eNOS at the Golgi complex

The localization of eNOS at the Golgi complex is mediated by myristoylation at its amino-terminus (Liu et al., 1996). Furthermore, the first 35 amino acid residues of eNOS are required and sufficient for the targeting of eNOS to the Golgi complex (Liu et al., 1997). Remarkably, the relevance of the presence of eNOS at the Golgi complex has remained a mystery. In contrast to eNOS that is localized at caveolae, it is not known whether Golgi-resident eNOS can be activated. Recently, it was shown that active eNOS can be found at the perinuclear region, but it is not clear whether this is due to activation in the Golgi or to intracellular trafficking of eNOS to the Golgi complex after activation at the plasma membrane (Fulton et al., 2002). Deletion of both palmitoylation sites in eNOS restricts eNOS localization to a perinuclear area, probably the Golgi complex (Garcia-Cardena et al., 1996), while eNOS activity is

largely impaired, suggesting that Golgi-localized eNOS cannot be activated. A crucial experiment to determine whether eNOS may be activated in the Golgi is to analyze activation of an eNOS molecule, in which all three acylation sites are replaced by a transmembrane domain and a Golgi retention motif. Yet this experiment remains to be awaited. If eNOS is able to become activated in the Golgi complex its regulation does not involve caveolin-1, since this eNOS regulator is not present in the same Golgi subcompartment as eNOS (Govers et al., 2002b). Since the Golgi most probably contains the largest pool of eNOS within the cell, it is certainly important to determine what the exact role of that pool is. Maybe the (inactive) Golgi pool of eNOS acts as a storage depot that can be easily translocated towards the plasma membrane for activation whenever that is required. However, the rather even distribution of eNOS over all Golgi stacks and TGN is not in favor of this and more likely suggests that eNOS routinely travels through the Golgi complex in an intracellular trafficking cycle, perhaps involving the plasma membrane and/or the cytosol (Govers et al., 2002b). This scenario is supported by FRAP (fluorescence recovery after photobleaching) studies that show that eNOS within the Golgi complex is not a silent pool of eNOS that does not move (Sowa et al., 1999). Other studies have suggested that upon activation, eNOS redistributes from the plasma membrane towards the Golgi complex, possibly via the cytoplasm (Goetz et al., 1999; Figueroa et al., 2002).

A protein that has been implicated in the regulation of eNOS' subcellular disribution is the NOS Interacting Protein NOSIP. NOSIP binds to the carboxy-terminus of the eNOS oxygenase domain, distal of the caveolin-1 binding motif. NOSIP and caveolin-1 are not likely to bind eNOS simultaneously, since a peptide corresponding to the caveolin-1 scaffolding domain inhibits eNOS-NOSIP complex formation (Dedio et al., 2001). NOSIP overexpression results in a decrease in the amount of eNOS at the plasma membrane, an increase at the Golgi complex, as well as in a reduced eNOS activity. Since NOSIP does not affect eNOS activity *in vitro* these results indicate that eNOS activation requires its presence at the plasma membrane rather than at the Golgi.

Cytosolic eNOS

As for the role of the Golgi localization of eNOS, it is also not known why a relatively small amount of eNOS is present in the cytosol and whether those eNOS molecules are active or activatable. All studies so far agree on the presence of eNOS in the cytosol, but there is some discussion on the questions 'how' and 'why'. It has been suggested that upon eNOS activation by agonists, eNOS dissociates from caveolin-1, binds calmodulin, and gets depalmitoylated, whereafter it is present in the cytosol, being able to produce NO (Michel et al., 1993; Prabhakar et al., 1998; Figueroa et al., 2002). However, not all research groups could detect this phenomenon (Liu et al., 1995; Zabel et al., 2002; L. Bevers and R. Govers, unpublished observations). Cytosolic eNOS is certainly without palmitate (Liu et al., 1995). In contrast, cytosolic eNOS still contains its myristoyl group as this modification is irreversible (Liu et al., 1995). This implies the presence of an additional eNOS-interacting protein which is able to cover the myristoyl group when eNOS is in its cytosolic form, in homology with the GDI factors for rab proteins which cover the rab geranylgeranyl group. Cytosolic eNOS is not likely to become activated by agonists. eNOS, in which the myristoylation site is removed, is completely cytosolic and still maintains its activity *in vitro*, but cannot be activated in intact cells (Sakoda et al., 1995; Feron et al., 1998b). In accordance, this mutant is hardly phosphorylated at Ser-1177 in response to VEGF (Gonzalez et al., 2002).

eNOS at cell-cell contacts

Recently, it was shown that eNOS is highly enriched at cell-cell contacts in cultures of both microvascular (capillary) and macrovascular (aortic) endothelial cells (Govers et al., 2002a). Its presence at these contact sites is dependent on their maturation as eNOS is not yet present at contact sites of early confluent monolayers. Moreover, it is independent of the formation of adherens junctions since VE-cadherin and plakoglobin-positive junctions already form before the cell-cell contacts become enriched in eNOS. Importantly, eNOS activity induced by agonists was shown to be highly correlative with its presence at cell-cell contacts.

The presence of eNOS at these sites has some major implications. First, (patho)physiological conditions that alter the organization of the endothelium may directly affect the enzymatic activity of eNOS. This has important biological relevance. NO is required for VEGF- and histamine-induced increases in endothelial permeability (Yuan et al., 1993; Fukumura et al., 2001). An increase in endothelial permeability, induced by these hormones, will break up cell-cell contacts and may provide an important negative feedback mechanism for eNOS, thus inhibiting further generation of NO and preventing major endothelial leakage. In addition, if the integrity of the endothelium is disturbed by bacteria or tumor metastases, disruption of endothelial contact sites will have a negative impact on eNOS-mediated NO generation. Subsequently, the diminished NO generation may induce the local expression of adhesion molecules at the luminal site of the endothelium, resulting in the attraction of leukocytes that will try to kill the pathogen (Khan et al., 1996; Adams et al., 1997).

Second, the localization of eNOS at cell-cell contacts, the limited working range of NO due to its short half-life, and the role of NO in the regulation of endothelial permeability has an obvious meaning. By being able to locally generate NO at the contact sites, eNOS is able to regulate these sites with high precision, without too much interference from NO scavenging

factors. This is very important since both low and high NO levels induce hyperpermeability of the endothelium, suggesting that the NO-mediated organization of the endothelial permeability involves a complex regulatory mechanism, that may consist of multiple NO-sensitive pathways (Baldwin et al., 1998; Fischer et al., 1999). This is consistent with the finding that low doses of the eNOS agonist estrogen decreases endothelial permeability, while high doses increase permeability (Cho et al., 1999).

Third, cell-cell contacts are important mechanosensors for fluid shear stress (Kano et al., 2000). eNOS is activated by fluid shear stress, suggesting that eNOS is in proximity of molecules that are involved in signaling fluid shear stress. PECAM-1, which is localized together with eNOS at contact sites, is involved in shear stress-induced cell activation and the regulation of endothelial permeability (Osawa et al., 2002; Mamdouh et al., 2003). Shear stress induces the tyrosine phosphorylation of PECAM-1, which is required for activation of downstream signaling pathways, including those involving MAP kinases. Both eNOS and PECAM-1 are in part Triton X-100-insoluble at cell-cell contacts, suggesting an interaction between these molecules and the cytoskeleton (Ayalon et al., 1994; Govers et al., 2002a). The role of the cytoskeleton in the maintenance of the endothelial permeability, and its sensitivity to NO, implicate a functional localization of the eNOS molecules that are linked to the cytoskeleton (Baldwin et al., 1998).

The receptor for the eNOS agonist VEGF is also localized at cell-cell contacts, where it becomes tyrosine phosphorylated during shear stress (Chen et al., 1999). In contrast to PECAM-1 and eNOS, the VEGF receptor is localized at adherens junctions where it is associated with VE-cadherin and acts as a mechanical transducer for fluid shear stress (Carmeliet et al., 1999; Shay-Salit et al., 2002). Moreover, VEGF-induced receptor activation results in phosphorylation of components of the VE-cadherin complex (Esser et al., 1998). Though eNOS and the VEGF receptor are not localized in the same structure, their proximal localization may suggest a direct regulation. In support of this hypothesis, recent studies have shown that the VEGF receptor is essential for shear stress-induced activation of eNOS (Jin et al., 2003). Interestingly, Rho family GTPases are involved in the regulation of cytoskeleton, cell-cell contacts and eNOS activity, suggesting a role for Rho in NO-dependent aspects of endothelial permeability (Takai et al., 1995; Fukata and Kaibuchi, 2001; Ming et al., 2002).

The eNOS-interacting protein NOSTRIN (eNOS TRaffick INducer) also localizes at cell-cell contacts (Zimmermann et al., 2002). NOSTRIN, identified as an eNOS interactor by yeast two-hybrid analysis, is a 58 kDa endothelial protein containing a cdc15 domain at its N-terminus and a SH3 domain at its C-terminus, that interacts with the eNOS oxygenase domain. Similarly to eNOS, NOSTRIN predominantly localizes to a perinuclear region in subconfluent cells and only localizes at the plasma membrane when cell cultures become confluent. In addition, NOSTRIN was found hardly soluble in detergent Triton X-100, suggesting the association of NOSTRIN with cytoskeletal elements, possibly resembling the presence of eNOS at detergent-insoluble cell-cell contacts (Govers et al., 2002a). This may be caused by NOSTRIN's cdc15 domain, since other proteins containing this domain have been implicated in cytoskeletal organization. Interestingly, overexpression of NOSTRIN in eNOS-expressing CHO cells causes a redistribution and inhibition of eNOS, implying an important role for NOSTRIN in cellular eNOS regulation (Zimmermann et al., 2002).

The vasodilator-stimulated phosphoprotein VASP, member of the Ena/VASP protein family (reviewed in Kwiatkowski et al., 2003), is also localized at cell-cell contacts. Here, it is involved in the organization of the subcortical actin network. VASP binds G-actin and induces actin filament formation (Walders-Harbeck et al., 2002). Interestingly, VASP can be activated by NO, via NO-mediated activation of guanylate cyclase and the subsequent activation of cGMP-dependent protein kinase (Sporbert et al., 1999). As for eNOS, VASP has also been identified as a substrate of PKA, one of the kinases that becomes activated by shear stress (Boo et al., 2002; Comerford et al., 2002). Of note is that Rho GTPases have been implicated to play a role in VASP function (Dutartre et al., 1996). These data suggest a close relation between eNOS and VASP function in the regulation of endothelial cytoskeleton and permeability.

In summary, eNOS is present at cell-cell contacts where it is involved in the regulation of endothelial permeability. Vice versa, endothelial cell-cell contacts are important for eNOS activity. It will be interesting to see whether pathophysiological conditions that decrease eNOS activity such as oxidized LDL (Blair et al., 1999), hyperglycemia (Noyman et al., 2002) and hypercholesterolemia (Feron et al., 1999) affect the localization of eNOS at cell-cell contacts.

eNOS in the nucleus

Over the last couple of years there have been occasional reports of eNOS residing in the nuclear compartment. After stimulation with VEGF eNOS has been reported to translocate from caveolae into the nucleus. Interestingly, this occurs in parallel with nuclear targeting of the VEGF receptor Flk-1/KDR and caveolin-1. It was speculated that nuclear translocation of caveolar eNOS and Flk-1/KDR may represent a mechanism for targeting NO production to the nuclear compartment where it might influence transcription factor activation (Feng et al., 1999). Moreover, eNOS has been observed to translocate into the nucleus of hepatocytes in states of various liver disorders (McNaughton et al., 2002), but the significance of this finding remains unclear. Again, nuclear translocation of eNOS might be due to growth factor activity, which characteristically is enhanced in chronic liver

inflammation and cirrhosis (Harada et al., 1999; Okano et al., 1999). Furthermore, nuclear eNOS has been reported for glandular elements of normal endometrium and endometrial carcinoma (Bentz et al., 1997) and brown adipocytes, where in addition it has been shown to be metabolically active. This lead to the suggestion of the existence of a noradrenaline-modulated functional NOS system in the nucleus of brown adipocytes, possibly involved in the modulation of gene expression (Giordano et al., 2002). Interestingly, nuclear NOS has also been demonstrated in cultured neonatal rat cardiomyocytes (iNOS; Buchwalow et al., 1997) and pancreatic ß-cells (nNOS; Lajoix et al., 2001), but taken together the body of evidence for nuclear localization of enzymatically active NOS is not as convincing as for other subcellular distribution patterns and the mechanism and control of nuclear transport as well as its physiological consequences remain to be defined.

nNOS

Several functions have been suggested for nNOS-mediated NO generation. In peripheral neurons, NO has an important role in neurotransmission, through a mechanism that most likely involves *S*-nitrosylation. In brain, NO has been implicated in several forms of synaptic plasticity. In skeletal muscle, nNOS-derived NO may play a role in dilating adjacent blood vessels (Thomas and Victor, 1998). This could serve to increase the blood flow to contracting skeletal muscles to support their enhanced metabolic needs. In the heart, sarcoplasmic reticulum-localized nNOS plays an important role in the autocrine regulation of myocardial contractility, which is thought to be mediated via NO-induced activation of Ca^{2+} channels in the sarcoplasmic reticulum (Ashley et al., 2002; Barouch et al., 2002).

In contrast to eNOS, nNOS as well as iNOS are not anchored in the membrane by lipid modifications. In addition, they are not regulated by Akt kinase (Fulton et al., 1999). Nevertheless, nNOS can be phosphorylated *in vivo*. Ca^{2+}/calmodulin-dependent kinase IIa phosphorylates nNOS at Ser-852 (human nNOS, Fig. 1), which inhibits its activity (Hayashi et al., 1999; Komeima et al., 2000). Remarkably, phosphorylation of eNOS at the corresponding serine residue (eNOS Ser-615, see figure 1), has a positive effect on its activity (Michell et al., 2002). Both nNOS and iNOS are degraded by the ubiquitin-proteasome system (Bender et al., 2000; Musial and Eissa, 2001). Like eNOS, nNOS is tightly regulated by calmodulin. Because NO reacts relatively non-specifically with cysteines to form S-nitrosothiols adducts thereby affecting protein function, anchoring nNOS to specific proteins or membranes may deliver neurally generated NO selectively to its physiological targets. nNOS is targeted to membranes via its amino-terminal PDZ (PSD-95/Discs-large/ZO-1) domain (Fig. 3). PDZ domains are small modular protein-protein interaction interfaces that play an essential role in gathering receptors, channels, and downstream effectors in protein complexes at cell junctions (Harris et al., 2001a). nNOS has an extended PDZ domain that can bind ligands in an unusual head-to-tail arrangement (Hillier et al., 1999; Harris et al., 2001a). The 30 amino acid residue extension comprises a rigid ß-hairpin finger structure that contains a near-consensus PDZ "pseudo-peptide" sequence (Wang et al., 2000). This extension can bind the PDZ domain of either PSD-95 or α1-syntrophin, leaving the true nNOS PDZ motif available for interaction with additional nNOS binding partners (Christopherson et al., 1999; Hillier et al., 1999).

In neurons, the nNOS PDZ domain links nNOS to membranes via its interaction with the postsynaptic density protein PSD-95 (Brenman et al., 1996), resulting in the presence of half of nNOS in the particulate cell fraction and in the enrichment of nNOS at synaptic junctions (Hecker et al., 1994) (Fig. 3A). PSD-95 contains three PDZ motifs that mediate the role of PSD-95 in protein clustering (Imamura et al., 2002). It is tempting to speculate that the PDZ domains of PSD-95 bring nNOS in direct contact with its downstream targets or regulators. Intriguingly, PSD-95 also interacts with the NMDA glutamate receptor, which is a potent activator of nNOS by its ability to induce a calcium influx (Kornau et al., 1995; Christopherson et al., 1999). In addition, the α2/ß1 isoform of NO target guanylyl cyclase also associates with membranes via PSD-95 (Russwurm et al., 2001; Zabel et al., 2002). This resembles the situation for eNOS, where multiple elements that are required for eNOS-mediated NO synthesis as well as NO targets are localized together with eNOS in caveolae. In brain, nNOS also interacts with α1-syntrophin (Hashida-Okumura et al., 1999) and with CtBP (Carboxyl-terminal-Binding Protein) (Riefler and Firestein, 2001). Whereas nNOS overexpression induces a redistribution of CtBP in transfected cells, the function of CtBP and the relevance of both nNOS-CtBP and nNOS-α1-syntrophin interactions in brain are currently unknown.

nNOS also binds through its PDZ domain to CAPON (Carboxy-terminally Associated Protein Of NOS) (Jaffrey et al., 2002). The latter links nNOS to Dexras1, a dexamethasone-induced G-protein of the Ras-family that is predominantly expressed in brain (Fang et al., 2000). Interestingly, the linkage to nNOS via CAPON is required for the sensitivity of Dexras1 for NO (Fang et al., 2000).

Members of the synapsin family, associated with synaptic vesicles and involved in their release, are also linked to nNOS via CAPON (Jaffrey et al., 2002). Intriguingly, in synapsin I and synapsin II knock-out mice, the cellular nNOS distribution is changed, suggesting an important role for synapsins in synaptic nNOS localization. Possibly, the nNOS-CAPON-synapsin complex formation is required for the role of nNOS in synaptic vesicle release, demonstrating the necessity of the close proximity of NOS and its target molecules (Meffert et al., 1994; Montague et al., 1994).

In skeletal muscle, nNOS is membrane-associated and enriched at motor endplates at the sarcolemma (Kobzik et al., 1994) (Fig. 3B). Here, nNOS localization is mediated by the PDZ-based interaction between nNOS and α1-syntrophin (Brenman et al., 1996; Adams et al., 2001). In skeletal muscle, α1-syntrophin acts as a molecular adaptor protein that recruits nNOS as well as other proteins to the dystrophin glycoprotein complex at the sarcolemma. nNOS molecules lacking the PDZ domain do not associate with skeletal muscle sarcolemma (Brenman et al., 1996). In accordance, the absence of dystrophin (as in Duchenne muscular dystrophy) or α1-syntrophin results in the redistribution of nNOS from the sarcolemma to the cytosol (Brenman et al., 1995; Kameya et al., 1999).

In skeletal muscle, nNOS also interacts through its PDZ domain with the calmodulin-dependent calcium pump PMCA4b (Schuh et al., 2001), and with caveolin-3 at sarcolemmal caveolae (Venema et al., 1997). Both interactions bring nNOS to the plasma membrane, while inhibiting its activity (Schuh et al., 2001; Sunada et al., 2001). Its localization at caveolae suggests that nNOS may be part of signaling pathways diverging from caveolae in skeletal muscle as is the case for eNOS in endothelium.

In addition, nNOS interacts with the 10 kDa protein PIN (protein inhibitor of nNOS) (Jaffrey and Snyder, 1996). In muscle, nNOS inhibitor PIN is localized in close proximity of the sarcolemma, thus aptly able to regulate nNOS activity there (Guo et al., 1999). nNOS and PIN also coexpress in brain, so perhaps PIN is regulating nNOS activity here as well (Roczniak et al.,

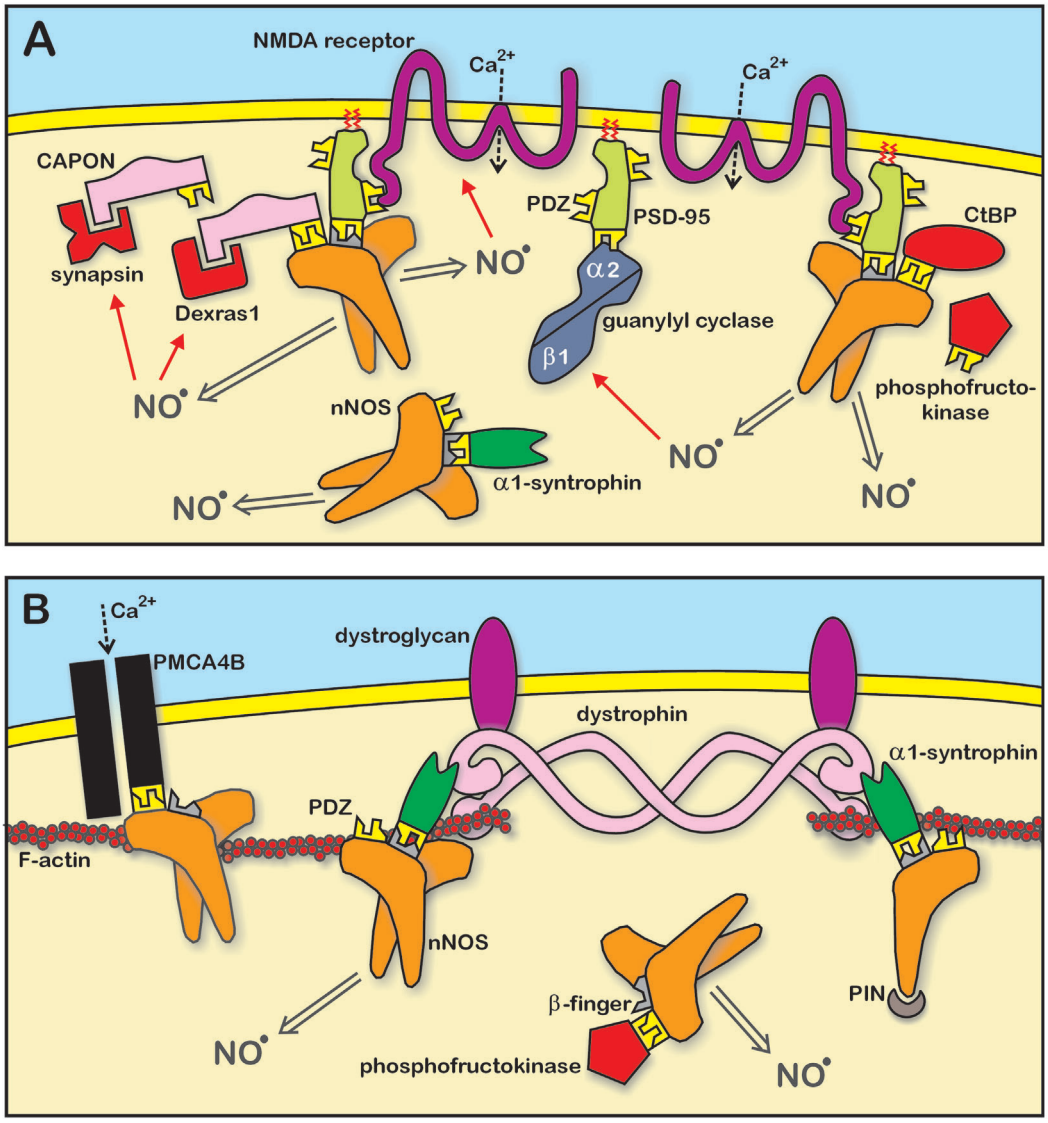


Fig. 3. A. Schematic representation of nNOS localisation at the neuronal junction. nNOS and the NO targets NMDA receptor and α2/ß1 guanylate cyclase are assembled at the plasma membrane via their interaction with PSD-95. Linkage of the NO-sensitive proteins synapsin and Dexras1 to this multiprotein complex involves adaptor protein CAPON. nNOS also interacts with CtBP and phosphofructokinase. PDZ domains are depicted in yellow, nNOS b-hairpin finger in grey (adapted from Christopherson et al., 1999). **B.** nNOS localisation at the skeletal muscle sarcolemma. The calcium channel PMCA4B and the dystroglycan-dystrophin complex target nNOS to the plasma membrane via PDZ motif-mediated interactions. The interaction with PIN abolishes nNOS activity. In the cytosol, nNOS interacts with phosphofructokinase (adapted from Brenman et al., 1996).

2000). PIN reduces nNOS activity by destabilizing the nNOS dimer, without altering its localization.

Another binding partner for nNOS in both brain and skeletal muscle is phosphofructokinase (Firestein and Bredt, 1999). Also this interaction is mediated by the PDZ domain of nNOS. In brain, phosphofructokinase and nNOS are enriched and interact in synaptic vesicle fractions, while in skeletal muscle, the interaction takes place in the cytosol.

In pancreatic beta-cells, nNOS binds via its PDZ domain to the receptor tyrosine phosphatase-like protein ICA512 (Ort et al., 2000). This interaction also brings nNOS to the membrane, since ICA512 is a transmembrane protein.

Other proteins found to interact with nNOS include hsp90, bradykinin B2 receptor, and NOSIP. The nNOS-hsp90 interaction is thought to enhance nNOS activity and/or decrease nNOS uncoupling (Bender et al., 1999; Song et al., 2002). However, the exact role of hsp90 in regulating nNOS activity *in vivo* remains to be established. The bradykinin B2 receptor binds both nNOS and eNOS (Ju et al., 1998; Golser et al., 2000). The bradykinin B2 receptor binds to the nNOS oxygenase domain, in close proximity to its catalytic center, where it blocks the electron transfer between the flavin and the heme. Several biological implications for the involvement of the bradykinin receptor in activation of nNOS have been suggested, including pain induction and penile erection (Golser et al., 2000). The interaction between nNOS and NOSIP has only been suggested from yeast two hybrid studies. nNOS appeared to bind NOSIP, though with a somewhat lower affinity than eNOS (Dedio et al., 2001). Whether this interaction exists *in vivo* and what the relevance of this interaction is remains to be established.

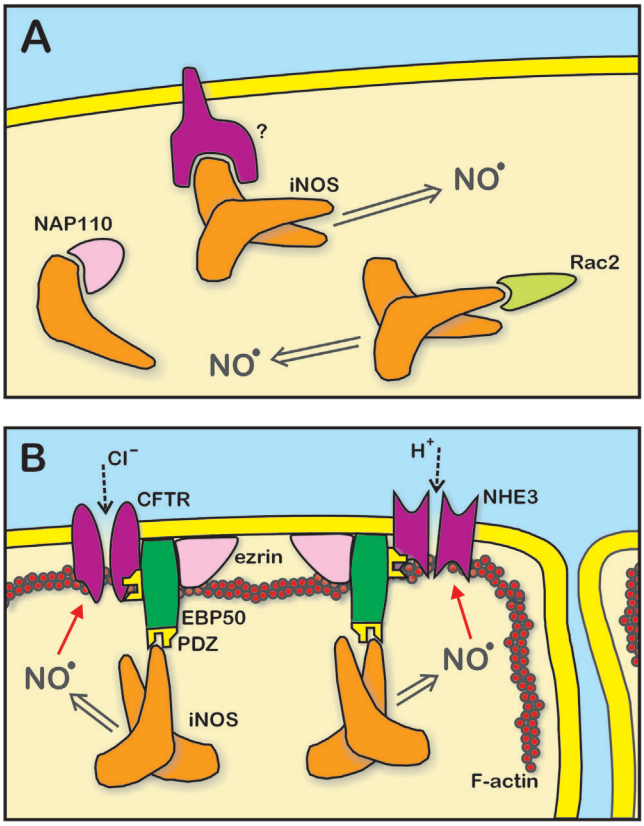


Fig. 4. Localization of iNOS in the macrophage **(A)** and epithelium **(B)**. In the macrophage, iNOS is partially membrane-associated via an unknown mechanism, possibly via interaction with a membrane-associated protein. iNOS binds to and is regulated by Rac2 and NAP110. In the enterocyte, iNOS is connected to the plasma membrane via its interaction with EBP50. EBP50 also interacts with the chloride channel CFTR and the Na^+/H^+-exchanger NHE3. Both CFTR and NHE3 are regulated by iNOS-generated NO.

iNOS

The inducible NOS enzyme was first discovered in mouse macrophages, but is now known to be expressed in a wide variety of cell types, in particular when activated by cytokines such as tumor necrosis factor-α, interferon-γ, and interleukin-1ß. Importantly, iNOS produces NO at micromolar levels, whereas both eNOS and nNOS produce NO in the nanomolar range. Hence, iNOS may not serve so much for signaling purposes but for the elimination of bacteria and tumor cells. An important feature of iNOS is its constitutive association with calmodulin. As a result, iNOS is always active, even when intracellular Ca^{2+} levels are low. This feature is at least in part caused by the absence of an autoinhibitory element in its primary structure (Nishida and de Montellano, 2001). In contrast to iNOS, both eNOS and nNOS contain such a domain, which causes their activity to be dependent on intracelular Ca^{2+} levels (Fig. 1).

It is currently unknown whether, as has been demonstrated for eNOS and nNOS, iNOS is regulated by phosphorylation. Despite the lack of a membrane anchor, in macrophages part of iNOS is found in membrane fractions (Forstermann et al., 1992) (Fig. 4A). However, the molecular basis of this membrane association is unclear at present. In macrophages, the iNOS oxygenase domain interacts with Rac2, a hematopoiesis-specific Rho GTPase (Kuncewicz et al., 2001). Rac2 may target iNOS to membranes since a small fraction of Rac2 is associated with membranes via its prenylation (Michaelson et al., 2001; Tao et al., 2002). Importantly, Rac2 overexpression induces a cellular redistribution as well as hyperactivation of iNOS, indicating that also for iNOS localization is important for its enzymatic activity. In macrophages, iNOS also interacts with NAP110 (NOS-associated protein of 110 kDa), which reduces iNOS activity by inhibiting iNOS dimer formation (Ratovitski et al., 1999b). NAP110 has been proposed to protect macrophages against NO toxicity until they are ready to kill. Hsp90, a binding partner for eNOS and nNOS, also interacts with iNOS. This interaction enhances iNOS activity in macrophages (Yoshida and

Xia, 2003). In specific parts of the brain, iNOS dimerization and activity may be regulated by the iNOS-interacting protein kalirin (Ratovitski et al., 1999a).

In skeletal muscle, where iNOS is expressed constitutively at low levels and most of the enzyme is membrane-associated, iNOS is associated with caveolin-3, the muscle-specific caveolin isoform (Gath et al., 1999). Both the expression and association with caveolin-3 is markedly enhanced upon stimulation with endotoxins or cytokines.

Caveolin-1 regulates degradation of iNOS by the proteasome, probably via inducing iNOS ubiquitination (Felley-Bosco et al., 2000; Kolodziejski et al., 2002). A small part of iNOS cofractionates with caveolin-1 in detergent-insoluble membrane fractions where iNOS degradation appears to take place. Nevertheless, despite the presence of a caveolin-1 binding motif in iNOS (similarly as in eNOS), and the ability of a peptide corresponding to the caveolin-1 scaffolding domain to inhibit iNOS activity *in vitro*, it is unclear at the moment whether caveolin-1 inhibits iNOS activity via a direct interaction *in vivo* (Garcia Cardena et al., 1997).

iNOS compartmentalization has also been described for epithelial cells (Fig. 4B). Within these cells, iNOS localizes to the apical domain in a submembranous protein complex that is tightly bound to cortical actin (Glynne et al., 2002). This localization is dependent on the C-terminus of iNOS which mediates the binding of the apical PDZ-containing protein EBP50 (ezrin-radixin-moesin-binding phosphoprotein 50), also known as NHERF (Na^+/H^+ exchanger regulatory factor). EBP50 is linked to the cortical actin via its interaction with the F-actin-binding protein ezrin (Reczek et al., 1997). The presence of multiple PDZ domains in EBP50 implicates a role for EBP50 in the formation of multiprotein complexes, in homology to the function of PSD-95 in neurons (see nNOS section). NO production in epithelial cells has been suggested to play a role in the regulation of apical membrane proteins such as the cystic fibrosis transmembrane conductance regulator CFTR and the renal proximal tubule epithelial Na^+/H^+ exchanger NHE3 (Glynne et al., 2002). Intriguingly, EBP50 interacts with both CFTR and NHE3 via its PDZ domains (Yun et al., 1997; Short et al., 1998; Shenolikar and Weinman, 2001), implicating that EBP50 acts as a molecular scaffold, enabling iNOS to locally regulate these proteins (Fig. 4B). In epithelial cells, iNOS may also be found at the tips of filopodia and at cytoplasmic vesicles, further delineating the role of iNOS compartmentalization (Markewitz et al., 1993). The role of iNOS in epithelium resembles, at least in part, the role of eNOS in endothelium. Exposure of intestinal epithelium to invasive bacteria or endotoxins increases the permeability of the epithelium in an NO-dependent fashion, resulting in a loss of enterocyte monolayer barrier function, suggesting an important role for iNOS in regulating epithelial permeability (Forsythe et al., 2002; Resta-Lenert and Barrett, 2002).

Concluding remarks

The function of NO synthases is not merely to generate NO. Their role is to produce the right amount of NO at the right place. Production of NO at the wrong place will result in S-nitrosylation and tyrosine nitration of proteins in an uncoordinated fashion and possibly in alteration of their function, while because of its high reactivity with proteins as well as with transition metals and superoxide, NO will not reach its prime targets. To circumvent this problem, NO synthases are localized at specific cellular sites, where they are in close proximity of their target molecules. This functional localization is defined by lipid modifications and protein-protein interactions. A perfect example of spatial confinement of NOS function has been demonstrated in heart where the specific localization of nNOS and eNOS in distinct intracellular compartments enables these enzymes to mediate their independent and opposite effects (Barouch et al., 2002), further elucidating the importance of targeted NOS localization.

Acknowledgements. R. Govers is supported by the Netherlands Heart Foundation (99.041) and the Netherlands Organization for Scientific Research (NWO; 902-26-224).

References

Abu-Soud H.M. and Stuehr D.J. (1993). Nitric oxide synthases reveal a role for calmodulin in controlling electron transfer. Proc. Natl. Acad. Sci. USA 90, 10769-10772.

Adams M.E., Mueller H.A. and Froehner S.C. (2001). In vivo requirement of the alpha-syntrophin PDZ domain for the sarcolemmal localization of nNOS and aquaporin-4. J. Cell. Biol. 155, 113-122.

Adams M.R., Jessup W., Hailstones D. and Celermajer D.S. (1997). L-arginine reduces human monocyte adhesion to vascular endothelium and endothelial expression of cell adhesion molecules. Circulation 95, 662-668.

Alonso J., Sanchez de Miguel L., Monton M., Casado S. and Lopez-Farre A. (1997). Endothelial cytosolic proteins bind to the 3' untranslated region of endothelial nitric oxide synthase mRNA: regulation by tumor necrosis factor alpha. Mol. Cell. Biol. 17, 5719-5726.

Andries L.J., Brutsaert D.L. and Sys S.U. (1998). Nonuniformity of endothelial constitutive nitric oxide synthase distribution in cardiac endothelium. Circ. Res. 82, 195-203.

Ashley E.A., Sears C.E., Bryant S.M., Watkins H.C. and Casadei B. (2002). Cardiac nitric oxide synthase 1 regulates basal and beta-adrenergic contractility in murine ventricular myocytes. Circulation 105, 3011-3016.

Aslan M., Ryan T.M., Townes T.M., Coward L., Kirk M.C., Barnes S., Alexander C.B., Rosenfeld S.S. and Freeman B.A. (2003). Nitric oxide-dependent generation of reactive species in sickle cell disease. Actin tyrosine nitration induces defective cytoskeletal polymerization. J. Biol. Chem. 278, 4194-4204.

Ayalon O., Sabanai H., Lampugnani M.G., Dejana E. and Geiger B. (1994). Spatial and temporal relationships between cadherins and

PECAM-1 in cell-cell junctions of human endothelial cells. J. Cell Biol. 126, 247-258.

Bae S.W., Kim H.S., Cha Y.N., Park Y.S., Jo S.A. and Jo I. (2003). Rapid increase in endothelial nitric oxide production by bradykinin is mediated by protein kinase A signaling pathway. Biochem. Biophys. Res. Commun. 306, 981-987.

Baldwin A.L., Thurston G. and Alnaemi H. (1998). Inhibition of nitric oxide synthesis increases venular permeability and alters endothelial actin cytoskeleton. Am. J. Physiol. 43, H1776-H1784.

Barouch L.A., Harrison R.W., Skaf M.W., Rosas G.O., Cappola T.P., Kobeissi Z.A., Hobai I.A., Lemmon C.A., Burnett A.L., O'Rourke B., Rodriguez E.R., Huang P.L., Lima J.A., Berkowitz D.E. and Hare J.M. (2002). Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature 416, 337-339.

Bender A.T., Silverstein A.M., Demady D.R., Kanelakis K.C., Noguchi S., Pratt W.B. and Osawa Y. (1999). Neuronal nitric-oxide synthase is regulated by the Hsp90-based chaperone system in vivo. J. Biol. Chem. 274, 1472-1478.

Bender A.T., Demady D.R. and Osawa Y. (2000). Ubiquitination of neuronal nitric-oxide synthase in vitro and in vivo. J. Biol. Chem. 275, 17407-17411.

Bentz B.G., Barnes M.N., Haines G.K., Lurain J.R., Hanson D.G. and Radosevich J.A. (1997). Cytoplasmic localization of endothelial constitutive nitric oxide synthase in endometrial carcinomas. Tumour Biol. 18, 290-300.

Blair A., Shaul P.W., Yuhanna I.S., Conrad P.A. and Smart E.J. (1999). Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. J. Biol. Chem. 274, 32512-32519.

Boo Y.C., Sorescu G., Boyd N., Shiojima I., Walsh K., Du J. and Jo H. (2002). Shear stress stimulates phosphorylation of endothelial nitric-oxide synthase at Ser1179 by Akt-independent mechanisms: role of protein kinase A. J. Biol. Chem. 277, 3388-3396.

Bouloumie A., Schinikerth V.B. and Busse R. (1999). Vascular endothelial growth factor up-regulates nitric oxide synthase expression in endothelial cells. Cardiovasc. Res. 41, 773-780.

Bredt D.S. and Snyder S.H. (1990). Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc. Natl. Acad. Sci. USA 87, 682-685.

Brenman J.E., Chao D.S., Xia H., Aldape K. and Bredt D.S. (1995). Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. Cell 82, 743-752.

Brenman J.E., Chao D.S., Gee S.H., McGee A.W., Craven S.E., Santillano D.R., Wu Z., Huang F., Xia H., Peters M.F., Froehner S.C. and Bredt D.S. (1996). Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. Cell 84, 757-767.

Brown G.C. (1995). Reversible binding and inhibition of catalase by nitric oxide. Eur. J. Biochem. 232, 188-191.

Bucci M., Gratton J.P., Rudic R.D., Acevedo L., Roviezzo F., Cirino G. and Sessa W.C. (2000). In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. Nat. Med. 6, 1362-1367.

Buchwalow I.B., Schulze W., Kostic M.M., Wallukat G. and Morwinski R. (1997). Intracellular localization of inducible nitric oxide synthase in neonatal rat cardiomyocytes in culture. Acta Histochem. 99, 231-240.

Cao S., Yao J. and Shah V. (2003). The Proline-rich domain of dynamin-2 is responsible for dynamin-dependent in vitro potentiation of endothelial nitric-oxide synthase activity via selective effects on reductase domain function. J. Biol. Chem. 278, 5894-5901.

Cao S., Yao J., McCabe T.J., Yao Q., Katusic Z.S., Sessa W.C. and Shah V. (2001). Direct interaction between endothelial nitric oxide synthase and dynamin-2: Implications for nitric oxide synthase function. J. Biol. Chem. 276, 14249-14256.

Carmeliet P., Lampugnani M.G., Moons L., Breviario F., Compernolle V., Bono F., Balconi G., Spagnuolo R., Oostuyse B., Dewerchin M., Zanetti A., Angellilo A., Mattot V., Nuyens D., Lutgens E., Clotman F., de Ruiter M.C., Gittenberger-de Groot A., Poelmann R., Lupu F., Herbert J.M., Collen D. and Dejana E. (1999). Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell 98, 147-157.

Caulin-Glaser T., Garcia-Cardena G., Sarrel P., Sessa W.C. and Bender J.R. (1997). 17 beta-estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca^{2+} mobilization. Circ. Res. 81, 885-892.

Chambliss K.L., Yuhanna I.S., Mineo C., Liu P., German Z., Sherman T.S., Mendelsohn M.E., Anderson R.G. and Shaul P.W. (2000). Estrogen receptor alpha and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. Circ. Res. 87, E44-E52.

Chen K.D., Li Y.S., Kim M., Li S., Yuan S., Chien S. and Shyy J.Y. (1999). Mechanotransduction in response to shear stress. Roles of receptor tyrosine kinases, integrins, and Shc. J. Biol. Chem. 274, 18393-18400.

Chen L., Daum G., Forough R., Clowes M., Walter U. and Clowes A.W. (1998). Overexpression of human endothelial nitric oxide synthase in rat vascular smooth muscle cells and in balloon-injured carotid artery. Circ. Res. 82, 862-870.

Chen Y., Panda K. and Stuehr D.J. (2002). Control of nitric oxide synthase dimer assembly by a heme-NO-dependent mechanism. Biochemistry 41, 4618-4625.

Cho M.M., Ziats N.P., Pal D., Utian W.H. and Gorodeski G.I. (1999). Estrogen modulates paracellular permeability of human endothelial cells by eNOS- and iNOS-related mechanisms. Am. J. Physiol. 45, C337-C349.

Christopherson K.S., Hillier B.J., Lim W.A. and Bredt D.S. (1999). PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. J. Biol. Chem. 274, 27467-27473.

Cines D.B., Pollak E.S., Buck C.A., Loscalzo J., Zimmerman G.A., McEver R.P., Pober J.S., Wick T.M., Konkle B.A., Schwartz B.S., Barnathan E.S., McCrae K.R., Hug B.A., Schmidt A.M. and Stern D.M. (1998). Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 91, 3527-3561.

Comerford K.M., Lawrence D.W., Synnestvedt K., Levi B.P. and Colgan S.P. (2002). Role of vasodilator-stimulated phosphoprotein in PKA-induced changes in endothelial junctional permeability. FASEB J. 16, 583-585.

Couet J., Sargiacomo M. and Lisanti M.P. (1997). Interaction of a receptor tyrosine kinase, EGF-R, with caveolins - Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. J. Biol. Chem. 272, 30429-30438.

Craig D.H., Chapman S.K. and Daff S. (2002). Calmodulin activates electron transfer through neuronal nitric-oxide synthase reductase domain by releasing an NADPH-dependent conformational lock. J. Biol. Chem. 277, 33987-33994.

Darby P.J., Kwan C.Y. and Daniel E.E. (2000). Caveolae from canine airway smooth muscle contain the necessary components for a role in Ca($^{2+}$) handling. Am. J. Physiol. 279, L1226-L1235.

D'Autreaux B., Touati D., Bersch B., Latour J.M. and Michaud-Soret I. (2002). Direct inhibition by nitric oxide of the transcriptional ferric uptake regulation protein via nitrosylation of the iron. Proc. Natl. Acad. Sci. USA 99, 16619-16624.

Dedio J., Konig P., Wohlfart P., Schroeder C., Kummer W. and Müller-Esterl W. (2001). NOSIP, a novel modulator of endothelial nitric oxide synthase activity. FASEB J. 15, 79-89.

Dimmeler S., Fleming I., Fisslthaler B., Hermann C., Busse R. and Zeiher A.M. (1999). Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399, 601-605.

Drab M., Verkade P., Elger M., Kasper M., Lohn M., Lauterbach B., Menne J., Lindschau C., Mende F., Luft F.C., Schedl A., Haller H. and Kurzchalia T.V. (2001). Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science 293, 2449-2452.

Dutartre H., Davoust J., Gorvel J.P. and Chavrier P. (1996). Cytokinesis arrest and redistribution of actin-cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42Hs. J. Cell. Sci. 109, 367-377.

Espey M.G., Thomas D.D., Miranda K.M. and Wink D.A. (2002). Focusing of nitric oxide mediated nitrosation and oxidative nitrosylation as a consequence of reaction with superoxide. Proc. Natl. Acad. Sci. USA 99, 11127-11132.

Esser S., Lampugnani M.G., Corada M., Dejana E. and Risau W. (1998). Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. J. Cell Sci. 111, 1853-1865.

Fang M., Jaffrey S.R., Sawa A., Ye K., Luo X. and Snyder S.H. (2000). Dexras1: a G protein specifically coupled to neuronal nitric oxide synthase via CAPON. Neuron 28, 183-193.

Felley-Bosco E., Bender F.C., Courjault-Gautier F., Bron C. and Quest A.F. (2000). Caveolin-1 down-regulates inducible nitric oxide synthase via the proteasome pathway in human colon carcinoma cells. Proc. Natl. Acad. Sci. USA 97, 14334-14339.

Feng Y.Y., Venema V.J., Venema R.C., Tsai N. and Caldwell R.B. (1999). VEGF induces nuclear translocation of Flk-1/KDR, endothelial nitric oxide synthase, and caveolin-1 in vascular endothelial cells. Biochem. Biophys. Res. Commun. 256, 192-197.

Feron O., Smith T.W., Michel T. and Kelly R.A. (1997). Dynamic targeting of the agonist-stimulated m2 muscarinic acetylcholine receptor to caveolae in cardiac myocytes. J. Biol. Chem. 272, 17744-17748.

Feron O., Dessy C., Opel D.J., Arstall M.A., Kelly R.A. and Michel T. (1998a). Modulation of the endothelial nitric-oxide synthase caveolin interaction in cardiac myocytes - Implications for the autonomic regulation of heart rate. J. Biol. Chem. 273, 30249-30254.

Feron O., Michel J.B., Sase K. and Michel T. (1998b). Dynamic regulation of endothelial nitric oxide synthase: complementary roles of dual acylation and caveolin interactions. Biochemistry 37, 193-200.

Feron O., Dessy C., Moniotte S., Desager J.P. and Balligand J.L. (1999). Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. J. Clin. Invest. 103, 897-905.

Figueroa X.F., Gonzalez D.R., Martinez A.D., Duran W.N. and Boric M.P. (2002). ACh-induced endothelial NO synthase translocation, NO release and vasodilatation in the hamster microcirculation in vivo. J. Physiol. 544, 883-896.

Firestein B.L. and Bredt D.S. (1999). Interaction of neuronal nitric-oxide synthase and phosphofructokinase-M. J. Biol. Chem. 274, 10545-10550.

Fischer S., Clauss M., Wiesnet M., Renz D., Schaper W. and Karliczek G.F. (1999). Hypoxia induces permeability in brain microvessel endothelial cells via VEGF and NO. Am. J. Physiol. 276, C812-C820.

Flam B.R., Hartmann P.J., Harrell-Booth M., Solomonson L.P. and Eichler D.C. (2001). Caveolar localization of arginine regeneration enzymes, argininosuccinate synthase, and lyase, with endothelial nitric oxide synthase. Nitric Oxide 5, 187-197.

Fleming I., Fisslthaler B., Dimmeler S., Kemp B.E. and Busse R. (2001). Phosphorylation of Thr(495) regulates Ca($^{2+}$)/calmodulin-dependent endothelial nitric oxide synthase activity. Circ. Res. 88, E68-E75.

Fontana J., Fulton D., Chen Y., Fairchild T.A., McCabe T.J., Fujita N., Tsuruo T. and Sessa W.C. (2002). Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. Circ. Res. 90, 866-873.

Forstermann U., Schmidt H.H., Kohlhaas K.L. and Murad F. (1992). Induced RAW 264.7 macrophages express soluble and particulate nitric oxide synthase: inhibition by transforming growth factor-beta. Eur. J. Pharmacol. 225, 161-165.

Forsythe R.M., Xu D.Z., Lu Q. and Deitch E.A. (2002). Lipopolysaccharide-induced enterocyte-derived nitric oxide induces intestinal monolayer permeability in an autocrine fashion. Shock 17, 180-184.

Fujimoto T. (1993). Calcium pump of the plasma membrane is localized in caveolae. J. Cell Biol. 120, 1147-1157.

Fukata M. and Kaibuchi K. (2001). Rho-family GTPases in cadherin-mediated cell-cell adhesion. Nat. Rev. Mol. Cell. Biol. 2, 887-897.

Fukumura D., Gohongi T., Kadambi A., Izumi Y., Ang J., Yun C.O., Buerk D.G., Huang P.L. and Jain R.K. (2001). Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. Proc. Natl. Acad. Sci. USA 98, 2604-2609.

Fulton D., Gratton J.P., McCabe T.J., Fontana J., Fujio Y., Walsh K., Franke T.F., Papapetropoulos A. and Sessa W.C. (1999). Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature 399, 597-601.

Fulton D., Fontana J., Sowa G., Gratton J.P., Lin M., Li K.X., Michell B., Kemp B.E., Rodman D. and Sessa W.C. (2002). Localization of endothelial nitric-oxide synthase phosphorylated on serine 1179 and nitric oxide in Golgi and plasma membrane defines the existence of two pools of active enzyme. J. Biol. Chem. 277, 4277-4284.

Garcia-Cardena G., Martasek P., Masters B.S., Skidd P.M., Couet J., Li S., Lisanti M.P. and Sessa W.C. (1997). Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. J. Biol. Chem. 272, 25437-25440.

Garcia-Cardena G., Oh P., Liu J., Schnitzer J.E. and Sessa W.C. (1996). Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. Proc. Natl. Acad. Sci. USA 93, 6448-6453.

Gath I., Ebert J., Godtel-Armbrust U., Ross R., Reske-Kunz A.B. and Forstermann U. (1999). NO synthase II in mouse skeletal muscle is associated with caveolin 3. Biochem. J. 340, 723-728.

Gewaltig M.T. and Kojda G. (2002). Vasoprotection by nitric oxide: mechanisms and therapeutic potential. Cardiovasc. Res. 55, 250-260.

Giordano A., Tonello C., Bulbarelli A., Cozzi V., Cinti S., Carruba M.O. and Nisoli E. (2002). Evidence for a functional nitric oxide synthase system in brown adipocyte nucleus. FEBS Lett. 514, 135-140.

Glynne P.A., Darling K.E., Picot J. and Evans T.J. (2002). Epithelial inducible nitric-oxide synthase is an apical EBP50-binding protein that directs vectorial nitric oxide output. J. Biol. Chem. 277, 33132-33138.

Goetz R.M., Thatte H.S., Prabhakar P., Cho M.R., Michel T. and Golan D.E. (1999). Estradiol induces the calcium-dependent translocation of endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. USA 96, 2788-2793.

Golser R., Gorren A.C., Leber A., Andrew P., Habisch H.J., Werner E.R., Schmidt K., Venema R.C. and Mayer B. (2000). Interaction of endothelial and neuronal nitric-oxide synthases with the bradykinin B2 receptor. Binding Of an inhibitory peptide to the oxygenase domain blocks uncoupled nadph oxidation. J. Biol. Chem. 275, 5291-5296.

Gonzalez E., Kou R., Lin A.J., Golan D.E. and Michel T. (2002). Subcellular targeting and agonist-induced site-specific phosphorylation of endothelial nitric-oxide synthase. J. Biol. Chem. 277, 39554-39560.

Govers R., Bevers L., de Bree P. and Rabelink T.J. (2002a). Endothelial nitric oxide synthase activity is linked to its presence at cell-cell contacts. Biochem. J. 361, 193-201.

Govers R., van der Sluijs P., van Donselaar E., Slot J.W. and Rabelink T.J. (2002b). Endothelial nitric oxide synthase and its negative regulator caveolin-1 localize to distinct perinuclear organelles. J. Histochem. Cytochem. 50, 779-788.

Gratton J.P., Fontana J., O'Connor D.S., Garcia-Cardena G., McCabe T.J. and Sessa W.C. (2000). Reconstitution of an endothelial nitric oxide synthase, hsp90 and caveolin-1 complex in vitro: Evidence that hsp90 facilitates calmodulin stimulated displacement of eNOS from caveolin-1. J. Biol. Chem. 275, 22268-22272.

Greif D.M., Kou R. and Michel T. (2002). Site-Specific Dephosphorylation of endothelial nitric oxide synthase by protein phosphatase 2A: evidence for crosstalk between phosphorylation Sites. Biochemistry 41, 15845-15853.

Gruetter C.A., Barry B.K., McNamara D.B., Kadowitz P.J. and Ignarro L.J. (1980). Coronary arterial relaxation and guanylate cyclase activation by cigarette smoke, N'-nitrosonornicotine and nitric oxide. J. Pharmacol. Exp. Ther. 214, 9-15.

Guo Y., Greenwood M.T., Petrof B.J. and Hussain S.N. (1999). Expression and regulation of protein inhibitor of neuronal nitric oxide synthase in ventilatory muscles. Am. J. Respir. Cell. Mol. Biol. 20, 319-326.

Guzik T.J., Mussa S., Gastaldi D., Sadowski J., Ratnatunga C., Pillai R. and Channon K.M. (2002). Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 105, 1656-1662.

Haasemann M., Cartaud J., Müller-Esterl W. and Dunia I. (1998). Agonist-induced redistribution of bradykinin B2 receptor in caveolae. J. Cell Sci. 111, 917-928.

Hanafy K.A., Krumenacker J.S. and Murad F. (2001). NO, nitrotyrosine, and cyclic GMP in signal transduction. Med. Sci. Monit. 7, 801-819.

Haqqani A.S., Kelly J.F. and Birnboim H.C. (2002). Selective nitration of histone tyrosine residues in vivo in mutatect tumors. J. Biol. Chem. 277, 3614-3621.

Harada K., Shiota G. and Kawasaki H. (1999). Transforming growth factor-alpha and epidermal growth factor receptor in chronic liver disease and hepatocellular carcinoma. Liver 19, 318-325.

Harris B.Z., Hillier B.J. and Lim W.A. (2001a). Energetic determinants of internal motif recognition by PDZ domains. Biochemistry 40, 5921-5930.

Harris M.B., Ju H., Venema V.J., Liang H., Zou R., Michell B.J., Chen Z.P., Kemp B.E. and Venema R.C. (2001b). Reciprocal phosphorylation and regulation of endothelial nitric-oxide synthase in response to bradykinin stimulation. J. Biol. Chem. 276, 16587-16591.

Hashida-Okumura A., Okumura N., Iwamatsu A., Buijs R.M., Romijn H.J. and Nagai K. (1999). Interaction of neuronal nitric-oxide synthase with alpha1-syntrophin in rat brain. J. Biol. Chem. 274, 11736-11741.

Hayashi Y., Nishio M., Naito Y., Yokokura H., Nimura Y., Hidaka H. and Watanabe Y. (1999). Regulation of neuronal nitric-oxide synthase by calmodulin kinases. J. Biol. Chem. 274, 20597-20602.

Haynes M.P., Li L., Sinha D., Russell K.S., Hisamoto K., Baron R., Collinge M., Sessa W.C. and Bender J.R. (2003). Src kinase mediates phosphatidylinositol 3-kinase/Akt-dependent rapid endothelial nitric-oxide synthase activation by estrogen. J. Biol. Chem. 278, 2118-2123.

Haynes M.P., Sinha D., Russell K.S., Collinge M., Fulton D., Morales-Ruiz M., Sessa W.C. and Bender J.R. (2000). Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the Pl3-kinase-Akt pathway in human endothelial cells. Circ. Res. 87, 677-682.

Hecker M., Mulsch A. and Busse R. (1994). Subcellular localization and characterization of neuronal nitric oxide synthase. J. Neurochem .62, 1524-1529.

Hess D.T., Matsumoto A., Nudelman R. and Stamler J.S. (2001). S-nitrosylation: spectrum and specificity. Nat. Cell Biol. 3, E1-E3.

Hillier B.J., Christopherson K.S., Prehoda K.E., Bredt D.S. and Lim W.A. (1999). Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex. Science 284, 812-815.

Imamura F., Maeda S., Doi T. and Fujiyoshi Y. (2002). Ligand binding of the second PDZ domain regulates clustering of PSD-95 with the Kv1.4 potassium channel. J. Biol. Chem. 277, 3640-3646.

Jaffrey S.R., Benfenati F., Snowman A.M., Czernik A.J. and Snyder S.H. (2002). Neuronal nitric-oxide synthase localization mediated by a ternary complex with synapsin and CAPON. Proc. Natl. Acad. Sci. USA 99, 3199-3204.

Jaffrey S.R., Erdjument-Bromage H., Ferris C.D., Tempst P. and Snyder S.H. (2001). Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat. Cell Biol. 3, 193-197.

Jaffrey S.R. and Snyder S.H. (1996). PIN: an associated protein inhibitor of neuronal nitric oxide synthase. Science 274, 774-777.

Jin Z.G., Ueba H., Tanimoto T., Lungu A.O., Frame M.D. and Berk B.C. (2003). Ligand-independent activation of vascular endothelial growth factor receptor 2 by fluid shear stress regulates activation of endothelial nitric oxide synthase. Circ. Res. 93, 354-363.

Ju H., Venema V.J., Marrero M.B. and Venema R.C. (1998). Inhibitory interactions of the bradykinin B2 receptor with endothelial nitric-oxide synthase. J. Biol. Chem. 273, 24025-24029.

Kameya S., Miyagoe Y., Nonaka I., Ikemoto T., Endo M., Hanaoka K., Nabeshima Y. and Takeda S. (1999). alpha1-syntrophin gene

disruption results in the absence of neuronal-type nitric-oxide synthase at the sarcolemma but does not induce muscle degeneration. J. Biol. Chem. 274, 2193-2200.

Kano Y., Katoh K. and Fujiwara K. (2000). Lateral zone of cell-cell adhesion as the major fluid shear stress-related signal transduction site. Circ. Res. 86, 425-433.

Khan B.V., Harrison D.G., Olbrych M.T., Alexander R.W. and Medford R.M. (1996). Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. Proc. Natl. Acad. Sci. USA 93, 9114-9119.

Knapp L.T., Kanterewicz B.I., Hayes E.L. and Klann E. (2001). Peroxynitrite-induced tyrosine nitration and inhibition of protein kinase C. Biochem. Biophys. Res. Commun. 286, 764-770.

Kobzik L., Reid M.B., Bredt D.S. and Stamler J.S. (1994). Nitric oxide in skeletal muscle. Nature 372, 546-548.

Kolodziejski P.J., Musial A., Koo J.S. and Eissa N.T. (2002). Ubiquitination of inducible nitric oxide synthase is required for its degradation. Proc. Natl. Acad. Sci. USA 99, 12315-12320.

Komeima K., Hayashi Y., Naito Y. and Watanabe Y. (2000). Inhibition of neuronal nitric-oxide synthase by calcium/ calmodulin-dependent protein kinase IIalpha through Ser847 phosphorylation in NG108-15 neuronal cells. J. Biol. Chem. 275, 28139-28143.

Kornau H.C., Schenker L.T., Kennedy M.B. and Seeburg P.H. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science 269, 1737-1740.

Kou R., Greif D. and Michel T. (2002). Dephosphorylation of endothelial nitric-oxide synthase by vascular endothelial growth factor. Implications for the vascular responses to cyclosporin A. J. Biol. Chem. 277, 29669-29673.

Kuncewicz T., Balakrishnan P., Snuggs M.B. and Kone B.C. (2001). Specific association of nitric oxide synthase-2 with Rac isoforms in activated murine macrophages. Am. J. Physiol. 281, F326-F336.

Kuzkaya N., Weissmann N., Harrison D.G. and Dikalov S. (2003). Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. J. Biol. Chem. 278, 22546-22554.

Kwiatkowski A.V., Gertler F.B. and Loureiro J.J. (2003). Function and regulation of Ena/VASP proteins. Trends Cell. Biol. 13, 386-392.

Lajoix A.D., Reggio H., Chardes T., Peraldi-Roux S., Tribillac F., Roye M., Dietz S., Broca C., Manteghetti M., Ribes G., Wollheim C.B. and Gross R. (2001). A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion. Diabetes 50, 1311-1323.

Lander H.M., Hajjar D.P., Hempstead B.L., Mirza U.A., Chait B.T., Campbell S. and Quilliam L.A. (1997). A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. J. Biol. Chem. 272, 4323-4326.

Laval F. and Wink D.A. (1994). Inhibition by nitric oxide of the repair protein, O6-methylguanine-DNA-methyltransferase. Carcinogenesis 15, 443-447.

Li S., Couet J. and Lisanti M.P. (1996). Src tyrosine kinases, Galpha subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. J. Biol. Chem. 271, 29182-29190.

Liu J., Garcia-Cardena G. and Sessa W.C. (1995). Biosynthesis and palmitoylation of endothelial nitric oxide synthase: mutagenesis of palmitoylation sites, cysteines-15 and/or -26, argues against depalmitoylation-induced translocation of the enzyme. Biochemistry 34, 12333-12340.

Liu J., Garcia-Cardena G. and Sessa W.C. (1996). Palmitoylation of endothelial nitric oxide synthase is necessary for optimal stimulated release of nitric oxide: implications for caveolae localization. Biochemistry 35, 13277-13281.

Liu J., Hughes T.E. and Sessa W.C. (1997). The first 35 amino acids and fatty acylation sites determine the molecular targeting of endothelial nitric oxide synthase into the Golgi region of cells: a green fluorescent protein study. J. Cell Biol. 137, 1525-1535.

Llovera M., Pearson J.D., Moreno C. and Riveros-Moreno V. (2001). Impaired response to interferon-gamma in activated macrophages due to tyrosine nitration of STAT1 by endogenous nitric oxide. Br. J. Pharmacol. 132, 419-426.

Lupas A., Van Dyke M. and Stock J. (1991). Predicting coiled coils from protein sequences. Science 252, 1162-1164.

Magee T., Fuentes A.M., Garban H., Rajavashisth T., Marquez D., Rodriguez J.A., Rajfer J. and Gonzalez-Cadavid N.F. (1996). Cloning of a novel neuronal nitric oxide synthase expressed in penis and lower urinary tract. Biochem. Biophys. Res. Commun. 226, 145-151.

Mamdouh Z., Chen X., Pierini L.M., Maxfield F.R. and Muller W.A. (2003). Targeted recycling of PECAM from endothelial surface-connected compartments during diapedesis. Nature 421, 748-753.

Mannick J.B., Schonhoff C., Papeta N., Ghafourifar P., Szibor M., Fang K. and Gaston B. (2001). S-Nitrosylation of mitochondrial caspases. J. Cell Biol. 154, 1111-1116.

Markewitz B.A., Michael J.R. and Kohan D.E. (1993). Cytokine-induced expression of a nitric oxide synthase in rat renal tubule cells. J. Clin. Invest. 91, 2138-2143.

Matsumoto A., Comatas K.E., Liu L. and Stamler J.S. (2003). Screening for nitric oxide-dependent protein-protein interactions. Science 301, 657-661.

McDonald K.K., Zharikov S., Block E.R. and Kilberg M.S. (1997). A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric-oxide synthase may explain the "arginine paradox". J. Biol. Chem. 272, 31213-31216.

McNaughton L., Puttagunta L., Martinez-Cuesta M.A., Kneteman N., Mayers I., Moqbel R., Hamid Q. and Radomski M.W. (2002). Distribution of nitric oxide synthase in normal and cirrhotic human liver. Proc. Natl. Acad. Sci. USA 99, 17161-17166.

McQuillan L.P., Leung G.K., Marsden P.A., Kostyk S.K. and Kourembanas S. (1994). Hypoxia inhibits expression of eNOS via transcriptional and posttranscriptional mechanisms. Am. J. Physiol. 267, H1921-H1927.

Meffert M.K., Premack B.A. and Schulman H. (1994). Nitric oxide stimulates $Ca^{(2+)}$-independent synaptic vesicle release. Neuron 12, 1235-1244.

Michaelson D., Silletti J., Murphy G., D'Eustachio P., Rush M. and Philips M.R. (2001). Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. J. Cell Biol. 152, 111-126.

Michel J.B., Feron O., Sacks D. and Michel T. (1997). Reciprocal regulation of endothelial nitric-oxide synthase by Ca^{2+}-calmodulin and caveolin. J. Biol. Chem. 272, 15583-15586.

Michel T., Li G.K. and Busconi L. (1993). Phosphorylation and subcellular translocation of endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. USA 90, 6252-6256.

Michell B.J., Harris M.B., Chen Z.P., Ju H., Venema V.J., Blackstone M.A., Huang W., Venema R.C. and Kemp B.E. (2002). Identification of regulatory sites of phosphorylation of the bovine endothelial nitric-oxide synthase at serine 617 and serine 635. J. Biol. Chem. 277, 42344-42351.

Ming X.F., Viswambharan H., Barandier C., Ruffieux J., Kaibuchi K., Rusconi S. and Yang Z. (2002). Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. Mol. Cell. Biol. 22, 8467-8477.

Molina y Vedia L., McDonald B., Reep B., Brune B., Di Silvio M., Billiar T.R. and Lapetina E.G. (1992). Nitric oxide-induced S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase inhibits enzymatic activity and increases endogenous ADP-ribosylation. J. Biol. Chem. 267, 24929-24932.

Montague P.R., Gancayco C.D., Winn M.J., Marchase R.B. and Friedlander M.J. (1994). Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. Science 263, 973-977.

Musial A. and Eissa N.T. (2001). Inducible nitric-oxide synthase is regulated by the proteasome degradation pathway. J. Biol. Chem. 276, 24268-24273.

Nathan C. (1997). Inducible nitric oxide synthase: what difference does it make? J. Clin. Invest. 100, 2417-2423.

Nishida C.R. and de Montellano P.R. (2001). Control of electron transfer in nitric-oxide synthases. Swapping of autoinhibitory elements among nitric-oxide synthase isoforms. J. Biol. Chem. 276, 20116-20124.

Noris M., Morigi M., Donadelli R., Aiello S., Foppolo M., Todeschini M., Orisio S., Remuzzi G. and Remuzzi A. (1995). Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. Circ. Res. 76, 536-543.

Noyman I., Marikovsky M., Sasson S., Stark A.H., Bernath K., Seger R. and Madar Z. (2002). Hyperglycemia reduces nitric oxide synthase and glycogen synthase activity in endothelial cells. Nitric Oxide 7, 187-193.

Ohta S., Komori K., Yonemitsu Y., Onohara T., Matsumoto T. and Sugimachi K. (2002). Intraluminal gene transfer of endothelial cell-nitric oxide synthase suppresses intimal hyperplasia of vein grafts in cholesterol-fed rabbit: a limited biological effect as a result of the loss of medial smooth muscle cells. Surgery 131, 644-653.

Oka N., Yamamoto M., Schwencke C., Kawabe J., Ebina T., Ohno S., Couet J., Lisanti M.P. and Ishikawa Y. (1997). Caveolin interaction with protein kinase C. Isoenzyme-dependent regulation of kinase activity by the caveolin scaffolding domain peptide. J. Biol. Chem. 272, 33416-33421.

Okano J., Shiota G. and Kawasaki H. (1999). Expression of hepatocyte growth factor (HGF) and HGF receptor (c-met) proteins in liver diseases: an immunohistochemical study. Liver 19, 151-159.

Ooboshi H., Toyoda K., Faraci F.M., Lang M.G. and Heistad D.D. (1998). Improvement of relaxation in an atherosclerotic artery by gene transfer of endothelial nitric oxide synthase. Arterioscler. Thromb. Vasc. Biol. 18, 1752-1758.

Ort T., Maksimova E., Dirkx R., Kachinsky A.M., Berghs S., Froehner S.C. and Solimena M. (2000). The receptor tyrosine phosphatase-like protein ICA512 binds the PDZ domains of beta2-syntrophin and nNOS in pancreatic beta-cells. Eur. J. Cell Biol. 79, 621-630.

Osawa M., Masuda M., Kusano K. and Fujiwara K. (2002). Evidence for a role of platelet endothelial cell adhesion molecule-1 in endothelial cell mechanosignal transduction: is it a mechanoresponsive molecule? J. Cell Biol. 158, 773-785.

Pagni M., Iseli C., Junier T., Falquet L., Jongeneel V. and Bucher P. (2001). trEST, trGEN and Hits: access to databases of predicted protein sequences. Nucleic Acids Res. 29, 148-151.

Pawloski J.R., Hess D.T. and Stamler J.S. (2001). Export by red blood cells of nitric oxide bioactivity. Nature 409, 622-626.

Pou S., Keaton L., Surichamorn W. and Rosen G.M. (1999). Mechanism of superoxide generation by neuronal nitric-oxide synthase. J. Biol. Chem. 274, 9573-9580.

Prabhakar P., Thatte H.S., Goetz R.M., Cho M.R., Golan D.E. and Michel T. (1998). Receptor-regulated translocation of endothelial nitric- oxide synthase. J. Biol. Chem. 273, 27383-27388.

Pritchard K.A. Jr., Ackerman A.W., Gross E.R., Stepp D.W., Shi Y., Fontana J.T., Baker J.E. and Sessa W.C. (2001). Heat shock protein 90 mediates the balance of nitric oxide and superoxide anion from endothelial nitric-oxide synthase. J. Biol. Chem. 276, 17621-17624.

Pritchard K.A., Ackerman A.W., Ou J., Curtis M., Smalley D.M., Fontana J.T., Stemerman M.B. and Sessa W.C. (2002). Native low-density lipoprotein induces endothelial nitric oxide synthase dysfunction: role of heat shock protein 90 and caveolin-1. Free Radic. Biol. Med. 33, 52-62.

Ratovitski E.A., Alam M.R., Quick R.A., McMillan A., Bao C., Kozlovsky C., Hand T.A., Johnson R.C., Mains R.E., Eipper B.A. and Lowenstein C.J. (1999a). Kalirin inhibition of inducible nitric-oxide synthase. J. Biol. Chem. 274, 993-999.

Ratovitski E.A., Bao C., Quick R.A., McMillan A., Kozlovsky C. and Lowenstein C.J. (1999b). An inducible nitric-oxide synthase (NOS)-associated protein inhibits NOS dimerization and activity. J. Biol. Chem. 274, 30250-30257.

Reczek D., Berryman M. and Bretscher A. (1997). Identification of EBP50: A PDZ-containing phosphoprotein that associates with members of the ezrin-radixin-moesin family. J. Cell Biol. 139, 169-179.

Resta-Lenert S. and Barrett K.E. (2002). Enteroinvasive bacteria alter barrier and transport properties of human intestinal epithelium: role of iNOS and COX-2. Gastroenterology 122, 1070-1087.

Riefler G.M. and Firestein B.L. (2001). Binding of neuronal nitric-oxide synthase (nNOS) to carboxyl-terminal-binding protein (CtBP) changes the localization of CtBP from the nucleus to the cytosol: a novel function for targeting by the PDZ domain of nNOS. J. Biol. Chem. 276, 48262-48268.

Robinson L.J., Busconi L. and Michel T. (1995). Agonist-modulated palmitoylation of endothelial nitric oxide synthase. J. Biol. Chem. 270, 995-998.

Roczniak A., Levine D.Z. and Burns K.D. (2000).Localization of protein inhibitor of neuronal nitric oxide synthase in rat kidney. Am. J. Physiol. 278, F702-F707.

Rodriguez J., Maloney R.E., Rassaf T., Bryan N.S. and Feelisch M. (2003). Chemical nature of nitric oxide storage forms in rat vascular tissue. Proc. Natl. Acad. Sci. USA 100, 336-341.

Russell K.S., Haynes M.P., Caulin-Glaser T., Rosneck J., Sessa W.C. and Bender J.R. (2000). Estrogen stimulates heat shock protein 90 binding to endothelial nitric oxide synthase in human vascular endothelial cells. Effects on calcium sensitivity and NO release. J. Biol. Chem. 275, 5026-5030.

Russwurm M., Wittau N. and Koesling D. (2001). Guanylyl cyclase/PSD-95 interaction: targeting of the nitric oxide-sensitive alpha2beta1 guanylyl cyclase to synaptic membranes. J. Biol. Chem. 276, 44647-

44652.

Rybin V.O., Xu X., Lisanti M.P. and Steinberg S.F. (2000). Differential targeting of beta -adrenergic receptor subtypes and adenylyl cyclase to cardiomyocyte caveolae. A mechanism to functionally regulate the cAMP signaling pathway. J. Biol. Chem. 275, 41447-41457.

Sakoda T., Hirata K., Kuroda R., Miki N., Suematsu M., Kawashima S. and Yokoyama M. (1995). Myristoylation of endothelial cell nitric oxide synthase is important for extracellular release of nitric oxide. Mol. Cell. Biochem. 152, 143-148.

Salt T.E., Zhang H., Mayer B., Benz B., Binns K.E. and Do K.Q. (2000). Novel mode of nitric oxide neurotransmission mediated via S-nitroso-cysteinyl-glycine. Eur. J. Neurosci. 12, 3919-3925.

Schlegel A., Schwab R.B., Scherer P.E. and Lisanti M.P. (1999). A role for the caveolin scaffolding domain in mediating the membrane attachment of caveolin-1 - The caveolin scaffolding domain is both necessary and sufficient for membrane binding in vitro. J. Biol. Chem. 274, 22660-22667.

Schubert W., Frank P.G., Woodman S.E., Hyogo H., Cohen D.E., Chow C.W. and Lisanti M.P. (2002). Microvascular hyperpermeability in caveolin-1 (-/-) knock-out mice. Treatment with a specific nitric-oxide synthase inhibitor, L-name, restores normal microvascular permeability in Cav-1 null mice. J. Biol. Chem. 277, 40091-40098.

Schuh K., Uldrijan S., Telkamp M., Rothlein N. and Neyses L. (2001). The plasmamembrane calmodulin-dependent calcium pump: a major regulator of nitric oxide synthase I. J. Cell Biol. 155, 201-206.

Sessa W.C., Garcia-Cardena G., Liu J., Keh A., Pollock J.S., Bradley J., Thiru S., Braverman I.M. and Desai K.M. (1995). The Golgi association of endothelial nitric oxide synthase is necessary for the efficient synthesis of nitric oxide. J. Biol. Chem. 270, 17641-17644.

Shaul P.W., Smart E.J., Robinson L.J., German Z., Yuhanna I.S., Ying Y., Anderson R.G. and Michel T. (1996). Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae. J. Biol. Chem. 271, 6518-6522.

Shay-Salit A., Shushy M., Wolfovitz E., Yahav H., Breviario F., Dejana E. and Resnick N. (2002). VEGF receptor 2 and the adherens junction as a mechanical transducer in vascular endothelial cells. Proc. Natl. Acad. Sci. USA 99, 9462-9467.

Shenolikar S. and Weinman E.J. (2001). NHERF: targeting and trafficking membrane proteins. Am. J. Physiol. 280, F389-F395.

Shi W., Wang X., Shih D.M., Laubach V.E., Navab M. and Lusis A.J. (2002a). Paradoxical reduction of fatty streak formation in mice lacking endothelial nitric oxide synthase. Circulation 105, 2078-2082.

Shi Y., Baker J.E., Zhang C., Tweddell J.S., Su J. and Pritchard K.A. Jr. (2002b). Chronic hypoxia increases endothelial nitric oxide synthase generation of nitric oxide by increasing heat shock protein 90 association and serine phosphorylation. Circ. Res. 91, 300-306.

Short D.B., Trotter K.W., Reczek D., Kreda S.M., Bretscher A., Boucher R.C., Stutts M.J. and Milgram S.L. (1998). An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. J. Biol. Chem. 273, 19797-19801.

Song Y., Cardounel A.J., Zweier J.L. and Xia Y. (2002). Inhibition of superoxide generation from neuronal nitric oxide synthase by heat shock protein 90: implications in NOS regulation. Biochemistry 41, 10616-10622.

Song Y., Zweier J.L. and Xia Y. (2001). Heat-shock protein 90 augments neuronal nitric oxide synthase activity by enhancing Ca^{2+}/calmodulin binding. Biochem. J. 355, 357-360.

Sowa G., Liu J., Papapetropoulos A., Rex-Haffner M., Hughes T.E. and Sessa W.C. (1999). Trafficking of endothelial nitric-oxide synthase in living cells. Quantitative evidence supporting the role of palmitoylation as a kinetic trapping mechanism limiting membrane diffusion. J. Biol. Chem. 274, 22524-22531.

Sporbert A., Mertsch K., Smolenski A., Haseloff R.F., Schonfelder G., Paul M., Ruth P., Walter U. and Blasig I.E. (1999). Phosphorylation of vasodilator-stimulated phosphoprotein: a consequence of nitric oxide and cGMP mediated signal transduction in brain capillary endothelial cells and astrocytes. Mol. Brain Res. 67, 258-266.

Stamler J.S., Jaraki O., Osborne J., Simon D.I., Keaney J., Vita J., Singel D., Valeri C.R. and Loscalzo J. (1992). Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc. Natl. Acad. Sci. USA 89, 7674-7677.

Stroes E., Kastelein J., Cosentino F., Erkelens W., Wever R., Koomans H., Luscher T. and Rabelink T. (1997). Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. J. Clin. Invest. 99, 41-46.

Sun J. and Liao J.K. (2002). Functional interaction of endothelial nitric oxide synthase with a voltage-dependent anion channel. Proc. Natl. Acad. Sci. USA 99, 13108-13113.

Sunada Y., Ohi H., Hase A., Hosono T., Arata S., Higuchi S., Matsumura K. and Shimizu T. (2001). Transgenic mice expressing mutant caveolin-3 show severe myopathy associated with increased nNOS activity. Hum. Mol. Genet. 10, 173-178.

Takahashi S. and Mendelsohn M.E. (2003). Synergistic activation of endothelial nitric-oxide synthase (eNOS) by HSP90 and Akt: calcium-independent eNOS activation involves formation of an HSP90-Akt-CaM-bound eNOS complex. J. Biol. Chem. 278, 30821-30827.

Takai Y., Sasaki T., Tanaka K. and Nakanishi H. (1995). Rho as a regulator of the cytoskeleton. Trends Biochem. Sci. 20, 227-231.

Tanimoto T., Jin Z.G. and Berk B.C. (2002). Transactivation of vascular endothelial growth factor (VEGF) receptor Flk-1/KDR is involved in sphingosine 1-phosphate-stimulated phosphorylation of Akt and endothelial nitric-oxide synthase (eNOS). J. Biol. Chem. 277, 42997-43001.

Tao W., Filippi M.D., Bailey J.R., Atkinson S.J., Connors B., Evan A. and Williams D.A. (2002). The TRQQKRP motif located near the C-terminus of Rac2 is essential for Rac2 biologic functions and intracellular localization. Blood 100, 1679-1688.

Thomas G.D. and Victor R.G. (1998). Nitric oxide mediates contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle. J. Physiol. 506, 817-826.

Vasquezvivar J., Kalyanaraman B., Martasek P., Hogg N., Masters B.S.S., Karoui H., Tordo P. and Pritchard K.A. (1998). Superoxide generation by endothelial nitric oxide synthase: The influence of cofactors. Proc. Natl. Acad. Sci. USA 95, 9220-9225.

Venema V.J., Ju H., Zou R. and Venema R.C. (1997). Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. J. Biol. Chem. 272, 28187-28190.

Venema R.C., Venema V.J., Ju H., Harris M.B., Snead C., Jilling T., Dimitropoulou C., Maragoudakis M.E. and Catravas J.D. (2003). Novel complexes of guanylate cyclase with heat shock protein 90 and nitric oxide synthase. Am. J. Physiol. (in press).

Walders-Harbeck B., Khaitlina S.Y., Hinssen H., Jockusch B.M. and Illenberger S. (2002). The vasodilator-stimulated phosphoprotein promotes actin polymerisation through direct binding to monomeric

actin. FEBS Lett. 529, 275-280.

Wang P., Zhang Q., Tochio H., Fan J.S. and Zhang M. (2000). Formation of a native-like beta-hairpin finger structure of a peptide from the extended PDZ domain of neuronal nitric oxide synthase in aqueous solution. Eur. J. Biochem. 267, 3116-3122.

Xu L., Eu J.P., Meissner G. and Stamler J.S. (1998). Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. Science 279, 234-237.

Yoshida M. and Xia Y. (2003). Heat shock protein 90 as an endogenous protein enhancer of inducible nitric oxide synthase. J. Biol. Chem. 278, 36953-36958.

Yuan Y., Granger H.J., Zawieja D.C., DeFily D.V. and Chilian W.M. (1993). Histamine increases venular permeability via a phospholipase C-NO synthase-guanylate cyclase cascade. Am. J. Physiol. 264, H1734-H1739.

Yun C.H., Oh S., Zizak M., Steplock D., Tsao S., Tse C.M., Weinman E.J. and Donowitz M. (1997). cAMP-mediated inhibition of the epithelial brush border Na+/H+ exchanger, NHE3, requires an associated regulatory protein. Proc. Natl. Acad. Sci. USA 94, 3010-3015.

Zabel U., Kleinschnitz C., Oh P., Nedvetsky P., Smolenski A., Muller H., Kronich P., Kugler P., Walter U., Schnitzer J.E. and Schmidt H.H. (2002). Calcium-dependent membrane association sensitizes soluble guanylyl cyclase to nitric oxide. Nat. Cell Biol. 4, 307-311.

Zimmermann K., Opitz N., Dedio J., Renne C., Müller-Esterl W. and Oess S. (2002). NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. USA 99, 17167-17172.

Zou M.H., Klein T., Pasquet J.P. and Ullrich V. (1998). Interleukin 1beta decreases prostacyclin synthase activity in rat mesangial cells via endogenous peroxynitrite formation. Biochem. J. 336, 507-512.

Zou M.H., Hou X.Y., Shi C.M., Nagata D., Walsh K. and Cohen R.A. (2002). Modulation by peroxynitrite of Akt- and AMP-activated kinase-dependent Ser1179 phosphorylation of endothelial nitric oxide synthase. J. Biol. Chem. 277, 32552-32557.

Accepted December 12, 2003