Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 82

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Nitric oxide (NO), a multifunctional second messenger, is implicated in physiological processes in mammals that range from immune response and potentiation of synaptic transmission to dilation of blood vessels and muscle relaxation. NO is a highly active molecule that diffuses across cell membranes and cannot be stored inside the producing cell. Its signaling capacity is controlled at the levels of biosynthesis and local availability. Its production by NO synthases is under complex and tight control, being regulated at transcriptional and translational levels, through co- and posttranslational modifications, and by subcellular localization. NO is synthesized from L-arginine by a family of nitric oxide synthases (NOS). Three NOS isoforms have been characterized: neuronal NOS (nNOS, NOS1) primarily found in neuronal tissue and skeletal muscle; inducible NOS (iNOS, NOS2) originally isolated from macrophages and later discovered in many other cell types; and endothelial NOS (eNOS, NOS3) present in vascular endothelial cells, cardiac myocytes, and in blood platelets. The enzymatic activity of all three isoforms is dependent on calmodulin, which binds to nNOS and eNOS at elevated intracellular calcium levels, while it is tightly associated with iNOS even at basal calcium levels. As a result, the enzymatic activity of nNOS and eNOS is modulated by changes in intracellular calcium levels, leading to transient NO production, while iNOS continuously releases NO independent of fluctuations in intracellular calcium levels and is mainly regulated at the gene expression level (Pacher et al. 2007).

The NOS enzymes share a common basic structural organization and requirement for substrate cofactors for enzymatic activity. A central calmodulin-binding motif separates an NH2-terminal oxygenase domain from a COOH-terminal reductase domain. Binding sites for cofactors NADPH, FAD, and FMN are located within the reductase domain, while binding sites for tetrahydrobiopterin (BH4) and heme are located within the oxygenase domain. Once calmodulin binds, it facilitates electron transfer from the cofactors in the reductase domain to heme enabling nitric oxide production. Both nNOS and eNOS contain an additional insert (40-50 amino acids) in the middle of the FMN-binding subdomain that serves as autoinhibitory loop, destabilizing calmodulin binding at low calcium levels and inhibiting electron transfer from FMN to the heme in the absence of calmodulin. iNOS does not contain this insert.

In this Reactome pathway module, details of eNOS activation and regulation are annotated. Originally identified as endothelium-derived relaxing factor, eNOS derived NO is a critical signaling molecule in vascular homeostasis. It regulates blood pressure and vascular tone, and is involved in vascular smooth
muscle cell proliferation, platelet aggregation, and leukocyte adhesion. Loss of endothelium derived NO is a key feature of endothelial dysfunction, implicated in the pathogenesis of hypertension and atherosclerosis. The endothelial isoform eNOS is unique among the nitric oxide synthase (NOS) family in that it is co-translationally modified at its amino terminus by myristylation and is further acylated by palmitoylation (two residues next to the myristoylation site). These modifications target eNOS to the plasma membrane caveolae and lipid rafts.

Factors that stimulate eNOS activation and nitric oxide (NO) production include fluid shear stress generated by blood flow, vascular endothelial growth factor (VEGF), bradykinin, estrogen, insulin, and angiopoietin. The activity of eNOS is further regulated by numerous post-translational modifications, including protein-protein interactions, phosphorylation, and subcellular localization.

Following activation, eNOS shuttles between caveolae and other subcellular compartments such as the noncaveolar plasma membrane portions, Golgi apparatus, and perinuclear structures. This subcellular distribution is variable depending upon cell type and mode of activation.

Subcellular localization of eNOS has a profound effect on its ability to produce NO as the availability of its substrates and cofactors will vary with location. eNOS is primarily particulate, and depending on the cell type, eNOS can be found in several membrane compartments: plasma membrane caveolae, lipid rafts, and intracellular membranes such as the Golgi complex.

**Literature references**


**Editions**

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eNOS activity is regulated by numerous post-translational modifications including phosphorylation and acylation, which also modulate its interactions with other proteins and its subcellular localization.

In general, following myristoylation and palmitoylation, eNOS localizes to caveolae in the plasma membrane, where in resting cells, it is bound to caveolin and remains inactive. Several agonists that raise intracellular calcium concentrations promote calmodulin binding to eNOS and the dissociation of caveolin from the enzyme, leading to an activated eNOS-calmodulin complex.

Phosphorylation plays a significant role in regulating eNOS activity, especially the phosphorylation of Ser1177, located within the reductase domain, which increases enzyme activity by enhancing reductase activity and calcium sensitivity. In unstimulated, cultured endothelial cells, Ser1177 is rapidly phosphorylated following a variety of stimuli: fluid shear stress, insulin, estrogen, VEGF, or bradykinin. The kinases involved in this process depend upon the stimuli applied. For instance, shear stress phosphorylates Ser1177 by activating Akt and PKA; insulin activates both Akt and the AMP-activated protein kinase (AMPK); estrogen and VEGF mainly phosphorylate eNOS via Akt; whereas the bradykinin-induced phosphorylation of Ser1177 is mediated by CaMKII. When Ser1177 is phosphorylated, NO production is increased several-fold above basal levels.

The phosphorylation of a threonine residue (Thr 495), located in the CaM binding domain, is associated with a decrease in eNOS activity. When this residue is dephosphorylated, substantially more CaM binds to eNOS and elevates enzyme activity. Stimuli associated with dephosphorylation of Thr945 (e.g., bradykinin, histamine, and Ca2+ ionophores) also increase Ca2+ levels resulting in the phosphorylation of Ser1177.

Additional phosphorylation sites, such as Ser114 and Ser633, and tyrosine phosphorylation have all been detected, but their functional relevance remains unclear. It is speculated that the tyrosine phosphorylation of eNOS is unlikely to affect enzyme activity directly, but more likely to impact the protein-protein interactions with associated scaffolding and regulatory proteins.
**Literature references**


**Editions**

2008-02-28  Reviewed  Enikolopov, G.
eNOS-interacting protein (NOSIP) is a 34-kDa nucleocytoplasmic shuttling protein that binds to the COOH-terminal region (amino acids 366-486) of the eNOS oxygenase domain. This protein association promotes translocation of eNOS from the plasma membrane caveolae to the cytoskeleton and inhibits eNOS activity. Studies have found that NOSIP accumulates in the cytoplasm specifically during the G2 phase of the cell cycle.

**Literature references**


**Editions**

2008-02-28 Reviewed Enikolopov, G.
NOSTRIN mediated eNOS trafficking

Location: Metabolism of nitric oxide: NOS3 activation and regulation

Stable identifier: R-HSA-203641

eNOS traffic inducer (NOSTRIN) is a novel 506-amino acid eNOS-interacting protein. Along with a decrease in eNOS activity, NOSTRIN causes translocation of eNOS from the plasma membrane to intracellular vesicular structures. NOSTRIN functions as an adaptor protein through homotrimerization and recruitment of eNOS, dynamin-2, and N-WASP to its SH3 domain. Studies indicated that NOSTRIN may facilitate vesicle fission and endocytosis of eNOS by coordinating the function of dynamin and N-WASP, which in turn, recruits the Arp2/3 complex, initiating actin filament polymerization. Overall, this process is thought to occur via caveolar endocytosis.

Literature references


Editions

2008-02-28 Reviewed Enikolopov, G.

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