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Review Article

Nitric oxide: A regulator of eukaryotic initiation factor 2 kinases

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ABSTRACT

Generation of nitric oxide ($NO^{\bullet}$) can upstream induce and downstream mediate the kinases that phosphorylate the α subunit of eukaryotic initiation factor 2 (eIF2α), which plays a critical role in regulating gene expression. There are four known eIF2α kinases (EIF2AKs), and $NO^{\bullet}$ affects each one uniquely. Whereas $NO^{\bullet}$ directly activates EIF2AK1 (HRI), it indirectly activates EIF2AK3 (PERK). EIF2AK4 (GCN2) is activated by depletion of L-arginine, which is used by nitric oxide synthase (NOS) during the production of $NO^{\bullet}$. Finally EIF2AK2 (PKR), which can mediate inducible NOS expression and therefore $NO^{\bullet}$ production, can also be activated by $NO^{\bullet}$. The production of $NO^{\bullet}$ and activation of EIF2AKs coordinately regulate physiological and pathological events such as innate immune response and cell apoptosis.

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Introduction

Nitric oxide ($NO^{\bullet}$) plays an important role in the control of physiological functions such as muscle relaxation and immune response [1,2]. In addition to its physiological significance, changes in $NO^{\bullet}$ concentration affect gene expression. One mechanism for $NO^{\bullet}$-mediated regulation of gene expression is via activation of multiple serine–threonine kinases that phosphorylate the eukaryotic initiation factor 2 (eIF2). When not phosphorylated, eIF2 initiates translation by forming an eIF2•TP•Met-tRNA$_i$ ternary complex, which promotes the binding of Met-tRNA$_i$ to the 40S ribosome–mRNA complex with the hydrolysis of GTP to GDP. To restart the cycle, the guanine exchange factor eIF2B must refresh the eIF2–GDP to eIF2–GTP [3]. Phosphorylation of Ser51 in the α subunit of eIF2 (eIF2α) stabilizes the eIF2–GDP–eIF2B complex, thus preventing the GDP–GTP exchange and halting translational initiation [4–6].

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The four identified serine–threonine eIF2α kinases (EIF2AKs) are (1) the heme-regulated inhibitor kinase (HRI, EIF2AK1), which responds to heme deprivation [7]; (2) the double-stranded RNA (dsRNA)-dependent protein kinase (PKR, EIF2AK2), which is activated by dsRNA produced during viral infection [8]; (3) PERK (EIF2AK3), which responds to the accumulation of unfolded protein response (UPR) in the endoplasmic reticulum (ER) [9,10]; and (4) GCN2 (EIF2AK4), which responds to amino acid depletion [11]. Whereas each EIF2AK is regulated specifically by its activator(s) and inhibitor(s), generation of NO• can be either an upstream activator (all four EIF2AKs) or a downstream mediator (PKR) of an EIF2AK-activated signaling pathway. This review discusses the mechanisms of the NO•-induced or -mediated EIF2AK signaling pathways and their physiological and pathological impacts.

The molecular mechanisms of NO•-activated or -mediated EIF2AK signaling pathways

Activation of HRI via the formation of a heme–Fe(II)NO• complex

HRI, as a hemoprotein, is activated by heme deficiencies through a series of *auto*phosphorylations [12–15]. Through sequence and mutagenesis analyses, an N-terminal hemin-binding domain (NT-HBD) containing a heme-binding His119 and a catalytic domain of HRI were identified [16,17]. NO• was first shown to activate HRI through binding with the recombinant NT-HBD [18]. It was suggested that the binding of NO• to the NT-HBD results in cleavage of the iron–histidyl bond to form a 5-coordinate ferrous nitrosyl [heme–Fe(II)NO] complex (Fig. 1A) [18]. However, additional evidence shows that the cleavage of the iron–histidyl bond is neither necessary nor sufficient for the activation of HRI by NO•[19,20]. The binding of NO• appears to disrupt the inhibitory interactions between the NT-HBD and the catalytic domain, thus activating HRI [20].

By using a recombinant N-terminal-deleted mutant and full-length HRI, two heme-binding sites in HRI, His119/120 in the N-terminal and Cys409 in the catalytic domain, were shown to form a complex with one heme, regardless of whether it was a hemin–Fe(III) or heme–Fe(II) [21,22]. Hemin–Fe(III) inhibits HRI by forming a stable 6-coordinate hemin–Fe(III)–HRI complex with Cys409 as one of axial ligands (Fig. 1B) [20–23]. In the presence of NO•, heme–Fe(III) can be reduced to heme–Fe(II) [24], which binds to NO• to form a 5-coordinate heme–Fe(II)NO• complex (Fig. 1A) and leads to HRI activation with a conformation change [21,23]. This model might potentially be affected by the phosphorylation states of HRI and the allosteric effect of eIF2 binding, which can affect the heme-binding affinity and HRI conformation [20,23].

Activation of PERK by NO• via two distinctive mechanisms

It is commonly accepted that the elevation of NO• leads to ER stress and results in PERK activation. Treating either differentiated or undifferentiated neuroblastoma cells with the NO• donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) was found to induce ER stress and PERK activation [25]. In addition, cytokines, such as IL-1, were found to induce ER stress by up-regulating the expression of inducible nitric oxide synthase (iNOS) and thus increasing the intercellular NO• level in chondrocytes and islets of both rats and humans. In both chondrocytes and β cells, PERK was activated by IL-1β in an iNOS-dependent manner because inhibiting iNOS resulted in a decreased expression of ER-stress-associated genes [26,27].

Two mechanisms have been proposed for NO•-induced PERK activation. The first is that NO• induces PERK activation by disrupting Ca^{2+} homeostasis in the ER. NO• inactivates the sarcoplasmic/ER Ca^{2+}-ATPases (SERCA) family proteins on the ER membrane, which are responsible for transporting cytosolic Ca^{2+} into the ER. Simultaneously, NO• activates the ryanodine receptor of Ca^{2+} release channels (RyR), which facilitates the release of Ca^{2+} from the ER into the cytosol. The inhibition of SERCA and activation of RyR lead to the depletion of Ca^{2+} in the ER and sequentially disrupt the protein-folding process, which increases ER stress and activates PERK [28,29] (Fig. 2A).

In addition to disrupting the Ca^{2+} channels, NO• also interrupts the flux of Ca^{2+} between the mitochondria and the ER [30], which are in close proximity [31–33]. The NO•-induced depletion of Ca^{2+} in the ER appears to be coupled to a mitochondrial Ca^{2+} influx [30,32,34]. When the Ca^{2+} released from the ER is collected in the matrix of the mitochondria, the mitochondrial membrane loses its potential. This depolarization disrupts the respiratory chain and increases production of reactive oxygen species (ROS), which further facilitates Ca^{2+} efflux from the ER [35] (Fig. 2A). In an attempt to alleviate ER stress, an elevation of NO• also stimulates an efflux of Ca^{2+} from mitochondria to ER, which activates the p90ATF6-mediated ER-stress response and protects cells from Ca^{2+} flux-caused damage [30].

The other proposed mechanism of NO•-induced PERK activation is through S-nitrosylation of protein disulfide isomerase (PDI), which facilities the folding of targeted proteins by either forming a disulfide bond or isomerizing a misfolded disulfide bond in the ER [36–38]. When cells were treated with a NO• donor, such as *S*-nitrosocysteine or O^2-[2,4-dinitro-5-(*N*-methyl-*N*-4-carboxyphenylamino)phenyl]1-(*N*,*N*-dimethylamino)diazen-1-ium-1,2-diolate, PDI was S-nitrosylated and inhibited. Accompanying the formation of S-nitrosylated PDI (SNO–PDI) and the loss of PDI activity, the activation of ER-stress-induced genes such as XBP-1, CCAAT-enhancer-binding protein (C/EBP)-homologous protein (CHOP), and PERK was detected [36,37]. It was proposed that when intracellular levels of NO• rise, NO• interacts with PDI on its thioredoxin domain, which forms one or two *S*-nitrosothiols. Furthermore, the two active thiols might share the NO• group and form a SNO2 group. After S-nitrosylation, the chaperone activity of PDI decreases, which leads to an accumulation of misfolded protein in the ER. This prolonged ER stress activates UPR and PERK [39] (Fig. 2B). It is worthwhile to notice that although SNO–PDI formation in NO• donor-treated cell lysate was detected by immunoblot and mass spectroscopy [36,37], the formation of SNO2–PDI is only a prediction based on an in silico study [40].

Production of NO• depletes L-Arg and induces GCN2 activity

Unlike NO•-induced HRI or PERK activation, GCN2 activity is not directly controlled by NO• but rather is regulated by NOS-catalyzed production of NO•. L-Arg is the substrate for all three NOSs, including iNOS and two constitutive NOSs (cNOSs), which convert L-Arg to NO• and L-citrulline [41–43]. Upon activation of NOS, cellular L-Arg levels begin to decrease, which eventually leads to L-Arg starvation, GCN2 activation, and eIF2α phosphorylation [44,45] (Fig. 3). To alleviate the demand for L-Arg in translation, L-Arg-sensitive GCN2 phosphorylates eIF2α, leading to the inhibition of protein synthesis, which also inhibits the translation of iNOS. The reduced expression of iNOS causes less L-Arg to be used to produce NO•, thus decreasing NO• levels and allowing L-Arg levels to build back up [44]. An additional effect of the depletion of L-Arg is the uncoupling of cNOSs and the generation of $O_2^{\bullet-}$, which then reacts with NO• to generate $ONOO^-$, resulting in further oxidative and ER stress. Thus, NOS-mediated GCN2 activation is often accompanied by PERK activation [45–47]. The NOS-mediated activation of GCN2 was based on the studies of cell culture conditions with limited supply of L-Arg [44,45]. The results may not apply to animal models, which have a constant supply of L-Arg. In fact, our recent study has indicated that the pattern of UVB-induced NO• release in mouse skin tissue is very different from that of cultured skin cells. The UVB-induced NO• production in cultured keratinocytes over time produces a bell-shaped curve with a sharp increase followed by a decrease to baseline [46], which may be the result of a lack of L-Arg. In mouse skin tissue, however, the UVB-induced NO• release remains a steady increase, indicating that the supply of L-Arg is not limited in the irradiated skin.