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Nitric Oxide: A Regulator of Eukaryotic Initiation Factor 2 Kinases

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Abstract

Generation of nitric oxide (NO[•]) can upstream induce and downstream mediate the kinases that phosphorylate the alpha subunit of eukaryotic initiation factor 2 (eIF2 α), which plays a critical role in regulating gene expression. There are four known eIF2 α kinases (EIF2AKs), and NO[•] affects each one uniquely. While NO[•] directly activates EIF2AK1 (HRI), it indirectly activates EIF2AK3 (PERK). EIF2AK4 (GCN2) is activated by depletion of L-arginine, which is used nitric oxide synthase (NOS) during the production of NO[•]. Finally EIF2AK2 (PKR), which can mediate inducible NOS expression and therefore NO[•] production, can also be activated by NO[•]. The production of NO[•] and activation of EIF2AKs coordinately regulate physiological and pathological events such as innate immune response and cell apoptosis.

Keywords

Nitric oxide; eukaryotic initiation factor 2; kinase; translation regulation; apoptosis

Introduction

Nitric oxide (NO[•]) 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, a change in NO[•] concentration impacts gene expression. One mechanism for NO[•]-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•GTP•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 alpha-subunit of eIF2 (eIF2 α) stabilizes the eIF2-GDP-eIF2B complex, thus preventing the GDP-GTP exchange and halting translational initiation [4–6].

The four identified serine-threonine eIF2 α kinases (EIF2AKs) are (1) the heme-regulated inhibitor kinase (HRI, EIF2AK1) that responds to heme deprivation [7], (2) the dsRNA-dependent protein kinase (PKR, EIF2AK2) that is activated by dsRNA produced during

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viral infection [8], (3) PERK (EIF2AK3), which responds to the accumulation of unfolded protein response (UPR) in the ER [9, 10], and (4) GCN2 (EIF2AK4), which responds to amino acid depletion [11]. While 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 will discuss the mechanisms for the NO[•] induced or mediated EIF2AK signaling pathways and their physiological and pathological impacts.

The Molecular Mechanisms for NO[•] Activated or Mediated EIF2AK Signaling Pathways

Activation of HRI via the formation of NO[•]-Fe(II)heme 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 showed 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 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 six-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 five-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 distinguish 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-acetyl-penicillamine (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 NOS (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-1 β in an iNOS-dependent manner since 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 Ca²⁺ homeostasis in ER. NO[•] inactivates the Sarco/ER Ca²⁺-ATPases (SERCA) family proteins on the ER membrane, which are responsible for transporting cytosolic Ca²⁺ into ER. Simultaneously, NO[•] activates the ryanodine receptor of Ca²⁺ release channels (RyR) that facilitates the release of Ca²⁺ from ER into cytosol. The inhibition of SERCA and activation of RyR leads to the depletion of Ca²⁺ in 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, $\text{NO}^{\bullet}$ also interrupts the flux of Ca^{2+} between the mitochondria and the ER [30], which are in close proximity [31–33]. The $\text{NO}^{\bullet}$ -induced depletion of Ca^{2+} in ER appears to be coupled with 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 ER [35] (Fig. 2A). In an attempt to alleviate ER stress, an elevation of $\text{NO}^{\bullet}$ 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 $\text{NO}^{\bullet}$ induced PERK activation is through S-nitrosylation of protein disulfide isomerase (PDI), which facilitates the folding of targeted proteins by either forming a disulfide bond or isomerizing a misfolded disulfide bond in ER [36–38]. When cells were treated with a $\text{NO}^{\bullet}$ donor, such as S-nitrosocysteine (SNOC) or *O*²-[2,4-dinitro-5-(*N*-methyl-*N*-4-carboxyphenylamino)phenyl]1-(*N,N*-dimethylamino)diazene-1-ium-1,2-diolate (PABA/ NO), 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, CHOP and PERK was detected [36, 37]. It was proposed that when intracellular levels of $\text{NO}^{\bullet}$ rise, $\text{NO}^{\bullet}$ interacts with PDI on its thioredoxin (Trx) domain, which forms one or two S-nitrosothiols. Furthermore, The two active thiols might share the $\text{NO}^{\bullet}$ group and form a SNO₂ group. After being S-nitrosylated, the chaperone activity of PDI decreases, which leads to an accumulation of misfolded protein in ER. This prolonged ER-stress activates UPR and PERK activation [39] (Fig. 2B). It is worthwhile to notice that while SNO-PDI formation in $\text{NO}^{\bullet}$ 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 $\text{NO}^{\bullet}$ depletes L-Arg and induces GCN2 activity

Unlike $\text{NO}^{\bullet}$ -induced HRI or PERK activation, GCN2 activity is not directly controlled by $\text{NO}^{\bullet}$ but rather is regulated by NOS-catalyzed production of $\text{NO}^{\bullet}$. L-Arg is the substrate for all three NOSs, including iNOS and two constitutive NOSs (cNOSs), which convert L-Arg to $\text{NO}^{\bullet}$ 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 eIF2 α phosphorylation [44, 45] (Fig. 3). In order to alleviate the demand for L-Arg in translation, L-Arg sensitive GCN2 phosphorylates eIF2 α , 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 $\text{NO}^{\bullet}$, thus decreasing $\text{NO}^{\bullet}$ levels and allowing L-Arg levels to build back up [44]. An additional effect of the depletion of L-Arg is the uncoupling of cNOS and the generation of $\text{O}_2^{\bullet-}$, which then reacts with $\text{NO}^{\bullet}$ to generate ONOO^- , resulting in further oxidative and ER-stress. Thus, NOS-mediated GCN2 activation is often accompanied with PERK activation [45–47]. The NOS-mediated activation of GCN2 was based on the studies of cell culture condition 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 for UVB-induced $\text{NO}^{\bullet}$ release in mouse skin tissue is very different from cultured skin cells. The UVB-induced $\text{NO}^{\bullet}$ 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 a result of a lack of L-Arg. In mouse skin tissue however, the UVB-induced $\text{NO}^{\bullet}$ release remains a steady increase, indicating that the supply of L-Arg is not limited in the irradiated skin.

PKR promotes NO[•] production through NF-κB

In addition to being an upstream regulator with an unknown mechanism [48], NO[•] production is regulated down-stream by PKR. The dsRNA-dependent activation of PKR results in an increased iNOS expression and therefore NO[•] elevation. The elevation in iNOS occurs through the activation of nuclear factor kappa B (NF-κB) [49], which is known to up-regulate the expression of iNOS [50]. Viral or synthetic dsRNA alone or in conjunction with interferon gamma (IFN-γ) increases the expression and activity of NF-κB, which induces iNOS expression and increase NO[•] production in a PKR dependent manner [49, 51]. In U373 MG astroglial cells, it was found that PKR activation was required for dsRNA-induced NF-κB activation and iNOS expression [51]. In airway epithelial cells, PKR knockouts lost the ability to induce NF-κB activation and iNOS expression by dsRNA [49]. Furthermore, PKR knockouts also showed reduced Interferon regulatory factor 1 (IRF-1) activity, which decreased the transcription of iNOS [49]. Based on the assumption that PKR is able to activate NF-κB through direct phosphorylation of the inhibitor of NF-κB (IκB) [52] or activation of IκB kinase (IKK) [53], a model for PKR-mediated induction of iNOS expression and NO[•] production is proposed (Fig. 4A).

While there is evidence showing that PKR mediates dsRNA-induced iNOS, the role of PKR in regulation of NO[•] production through iNOS is a subject of debate. Additional data indicates that iNOS induction occurs prior to PKR activation and is independent of functional PKR in cells infected with encephalomyocarditis virus (EMCV), a RNA virus known to induce PKR activation [54]. Moreover, the dsRNA-induced degradation of IκB and translocation of NF-κB into nucleus is also independent of PKR. In PKR knockout mice islet cells, dsRNA was still able to induce iNOS expression through activating NF-κB [55]. These controversial results could be due to tissue specific signaling for dsRNA-induced iNOS expression [51]. Two different signaling pathways are proposed for mediating the dsRNA-induced expression of iNOS. One is a PKR-dependent activation of NF-κB signaling pathway and the other is p38MAPK-dependent but PKR-independent CCAAT-enhancer-binding proteins β (C/EBPβ) signaling pathway [51, 54]. The proposed model is based on the assumption that if activities of both PKR and p38MAPK are diminished, iNOS is no longer inducible by dsRNA [51].

The Physiological Impacts of the Coordinative Effects of NO[•]-EIF2AKs

The role of NO[•]-HRI interaction in regulation of cell cytostasis and Hg²⁺ toxicity

The NO[•]-induced activation of HRI increases eIF2α phosphorylation and inhibits protein synthesis in nonerythroid cells. Since eIF2 plays an important role in the regulation of cell growth and death, it has been suggested that HRI may contribute to the NO[•]-regulated cell growth, differentiation, and apoptosis [18]. Furthermore, it has been found that HRI plays a role in mediating NO[•]-induced translation inhibition in breast cancer cells [48]. In invasive breast tumors, the activities of both cNOS and iNOS are higher than in benign or normal breast tissues [56]. Exposure of breast cancer cells to 1 mM diethylenetriamine-NONOate (DETA-NONOate), which releases NO[•] to a constant concentration of 0.5 μM [57], activated HRI and led to a gradual decrease in short half-life proteins and cell growth arrest. While the translational inhibition and cell cytostasis caused by exposure to 0.5 μM level of NO[•] was reversible, the effects of higher NO[•] level were not. Exposure of breast cancer cells to 2 mM DETA-NONOate resulted in the activation of both HRI and PKR and caused a sharp decrease in both short and long half-life proteins [48].

In addition to promoting cytostasis, the NO[•]-HRI interaction has been suggested to play a role in regulating Hg²⁺-induced cytotoxicity [58]. Metal cations, such as Hg²⁺, Cd²⁺, Zn²⁺ and Pb²⁺, inhibit HRI *in vitro* with their IC₅₀ values ranging from 0.6–8.5 μM. The

inhibition of HRI by Hg^{2+} , but not other heavy metal ions, was reversed by $\text{NO}^\bullet$ at the micromole level [58]. A potential mechanism for the metal cations-induced inhibition of HRI is that the cations compete with the heme in binding Cys or His and inhibits HRI by forming an inactive Mt^{2+} -HRI complex (Fig. 1C). While all these metal cations can potentially interact with His residues, there is a unique interaction between Hg^{2+} and HRI through the formation of Hg^{2+} -thiol bond with cysteine residues, including the Cys409, one of the axial ligands. It was proposed that $\text{NO}^\bullet$ reverses Hg^{2+} inhibited HRI by nitrosylating Cys409, likely to form a thiol adduct, S-NO in active site (Fig. 1C). It has thus been suggested that the $\text{NO}^\bullet$ and Hg^{2+} competitively control protein synthesis in cells, which may be important for cell survival under mercury contamination [58]. However, while the binding of Hg^{2+} to Cys on HRI was detected, the formation of HRI-S-NO was based only on the previous studies indicating that Cys can be S-nitrosylated by $\text{NO}^\bullet$ [59, 60]. Another possibility is that $\text{NO}^\bullet$ restores the activity of Hg^{2+} -inhibited HRI simply by reducing Hg^{2+} to Hg^+ , which is then released from HRI. $\text{Hg}^{2+}/\text{Hg}^+$ has a relatively higher standard reduction potential and is the only one of these tested metals that can be reduced by $\text{NO}^\bullet$ [58] (Table I).

The role of PERK in mediating $\text{NO}^\bullet$ -induced apoptosis

$\text{NO}^\bullet$ induces apoptosis by depleting Ca^{2+} in the ER, which increase ER-stress and activates PERK [61, 62]. The pro-apoptotic role of PERK comes from its ability to induce expression of the apoptotic protein, C/EBP-homologous protein (CHOP) via phosphorylation of eIF2 α and activation of activating transcription factor 4 (ATF4) [61–64]. PERK, along with the ER-stress-induced proteins inositol-requiring enzyme-1 (IRE1) and ATF6, helps mediate the $\text{NO}^\bullet$ -induced expression of CHOP through multiple signaling pathways [61–63]. The discovery that CHOP knockout β cells are more resistant to $\text{NO}^\bullet$ -induced apoptosis suggests that ER-stress-induced signaling pathways are responsible for $\text{NO}^\bullet$ -mediated β cell death [62]. The involvement of ER-stress-induced signaling pathways in $\text{NO}^\bullet$ -induced cell death was also observed in other cell lines including microglial cells [65, 66], macrophages [63], and neuronal cells [67] (Fig. 2C).

While $\text{NO}^\bullet$ -induced and ER-stress-mediated PERK activation increases CHOP expression [63], it is still debate whether PERK catalyzed eIF2 α phosphorylation is the cause of or just occurs simultaneously with $\text{NO}^\bullet$ -induced apoptosis. In chondrocytes, the $\text{NO}^\bullet$ donor SNAP increased CHOP expression and cell apoptosis but the $\text{NO}^\bullet$ inducer IL-1 did not [26], suggesting that SNAP may induce apoptosis via a $\text{NO}^\bullet$ -independent mechanism. Due to complex chemical structures, $\text{NO}^\bullet$ donors can modulate cellular processes in mechanisms beyond $\text{NO}^\bullet$ donation [68]. Additionally, cells overexpressing a dominant negative PERK or a PERK knockout showed no increase in cytokine-induced cell death but were more susceptible to the tunicamycin-induced cell death. This implies that $\text{NO}^\bullet$ is not necessary for PERK mediated apoptosis since tunicamycin-induced cell death is ER-stress dependent and $\text{NO}^\bullet$ independent [27]. It has also been suggested that instead of mediating cell death, the $\text{NO}^\bullet$ -induced UPR may play a protective role in cytokine induced apoptosis. β cells with a knockout PERK or a knockin nonphosphorylatable eIF2 α , eIF2 α S51A, are more susceptible to ER-stress [69, 70]. Also, during $\text{NO}^\bullet$ -induced apoptosis, the ER-stress response protein p90ATF6 is digested into p50ATF6, which activates the CHOP gene in the nucleus [63]. In addition to activating the CHOP apoptosis pathway, p50ATF6 activates the ER-stress-induced chaperon protein GRP78, which attenuates CHOP-induced apoptosis and promotes cell recovery [62] (Fig 2C).

Potential roles of PERK in $\text{NO}^\bullet$ -mediated physiological responses

$\text{NO}^\bullet$ -induced activation of PERK helps regulate Ca^{2+} flux and therefore affects cardiac and skeleton muscle contraction. While $\text{NO}^\bullet$ regulates muscle contraction by inducing ER Ca^{2+}

efflux via activation of Ca^{2+} channels and by inhibiting ER Ca^{2+} influx via inactivation of Ca^{2+} -ATPases, the resulting Ca^{2+} depletion in the ER leads to ER-stress and PERK activation [28, 29, 71]. Through a currently uncharacterized mechanism, the activated PERK reduces ER Ca^{2+} efflux and thus relieves ER stress and maintains the integrity of ER [29]. The $\text{NO}^{\bullet}$ -induced and PERK-mediated protection of ER integrity may also protect brain tissue from damage caused by ischemia and reperfusion (I/R) [72]. Treating brain tissue with the NOS inhibitor L-NAME reduced PERK activation and eIF2 α phosphorylation. These treated cells showed an increase in brain tissue damage from I/R, suggesting that translation inhibition protects brain cells from I/R-induced damage. However, the role of PERK in protecting cells from I/R-induced damage is limited to the brain since L-NAME treatments protected kidney and liver cells from damage without inducing eIF2 α phosphorylation [25] (Fig. 2C).

The long-acting $\text{NO}^{\bullet}$ donor diethylenetriamine (DETA)-NONOate induces PERK activation and eIF2 α phosphorylation. Additionally it is able to preferentially induce macrophages apoptosis in plaques without affecting normal smooth muscle cells or circulating macrophages/monocytes. The removal of macrophages from a plaque affects its stability decreases the risk of cardiovascular disease. A proposed explanation for the selectivity of $\text{NO}^{\bullet}$ -induced apoptosis is that plaque macrophages are more metabolically active and thus more sensitive to protein synthesis inhibition. As a result, DETA-NONOate can potentially be used for treatment of atherosclerotic plaques because it specifically targets plaque macrophages [73] (Fig. 2C).

Cell cycle regulation mediated by $\text{NO}^{\bullet}$ -induced activation of GCN2/PERK

$\text{NO}^{\bullet}$ production without a continuous supply of L-Arg can activate GCN2, which coordinates with PERK to regulate the cell cycle upon UVB-irradiation. Activation of GCN2 and PERK promotes UVB-induced G_1 arrest since a knockout of either GCN2 or PERK allowed cells to shift from the G_1 phase to the G_2 phase. Interestingly, elimination of eIF2 α phosphorylation by knocking in the nonphosphorylatable eIF2 α S51A prevented UVB-induced cell cycle shift, which suggests that PERK and GCN2 regulate the mammalian cell cycle via an eIF2 α phosphorylation independent pathway [74].

The cellular responses to $\text{NO}^{\bullet}$ production and PKR activation

PKR activation appears to be critical for an innate immune response to viral infections. Innate immune response cells, such as monocytes and macrophage, respond to pathogens by activating a Toll-like receptor (TLR) signaling cascade. DsRNA or single stranded DNA (ssDNA) interacts with TLR3 and activates an antiviral pathway by increasing the expression of iNOS and thus the production of $\text{NO}^{\bullet}$ [75]. However, the TLR3 mediated elevation of $\text{NO}^{\bullet}$ in monocytes or macrophages in response to dsRNA or ssDNA is diminished when the cells are treated with a PKR pharmacological inhibitor, which inhibits the ATP-binding site of PKR. This suggests that PKR is non-dispensable for virus-induced innate immune response (Fig. 4B).

PKR also plays a role in lipopolysaccharide (LPS)-induced inflammatory response by mediating the activation of the NF- κ B-STAT1-iNOS cascade [76]. In both microglial and astrocyte cells, PKR was activated within 5 min and was followed by STAT1 activation at 2 h post-LPS treatment. Reducing PKR activity by PKR-specific siRNA or 2-AP reduced NF- κ B activation, IFN- β production, STAT1 activation, iNOS expression, and $\text{NO}^{\bullet}$ production after LPS treatment. The PKR-mediated inflammatory response appears to be LPS specific since ganglioside-stimulated STAT1 phosphorylation is independent of PKR activation [76] (Fig. 4B).

Besides phosphorylating eIF2 α and inhibiting protein synthesis, PKR also promotes protein degradation via activation of the iNOS-p38MAPK pathway. Tumor necrosis factor α (TNF α) or interferon γ (INF γ) induces the NO $^{\bullet}$ -mediated activation of p38MAPK, which promotes muscle protein degradation in myotubes by increasing ROS production. Inhibition of PKR, p38MAPK, or iNOS in myoblasts attenuates ROS production and protects proteins from degradation. This suggests that NO $^{\bullet}$ production regulates protein degradation and is important in decreasing the overall protein concentration in a cell, though the mechanism for the ROS formation is not known [77] (Fig. 4B).

While activation of PKR increases cytosolic NO $^{\bullet}$ production, a higher level of NO $^{\bullet}$ produced by 2 mM DETA-NONate can also activate PKR, which along with HRI inhibits protein synthesis [48] as we discussed earlier. Interestingly, NO $^{\bullet}$ -induced and PKR-mediated translation inhibition appears more effective on cancer cells than on normal cells. PKR autophosphorylation is induced by DETA-NONOate in breast cancer cells but not in normal mammary epithelial cells. It appears that inactive PKR exists as a monomer in cancer cells, but is bound with its inhibitor p58 in normal cells [78, 79]. NO $^{\bullet}$ is only able to interact and activate monomeric PKR, but not the PKR-p58 heterodimer. Thus, DETA-NONOate has the potential to be used as an anti-cancer drug with limited side effect on healthy cells [48, 80] (Fig. 4B).

Conclusion

Via different mechanisms, NOS-catalyzed NO $^{\bullet}$ production regulates the activation of the four EIF2AKs, and thus inhibits protein synthesis. Conversely, activation of EIF2AK2 (PKR) increases iNOS expression and thus NO $^{\bullet}$ production. Coordination between NO $^{\bullet}$ production and EIF2AK activation is well regulated. A shift of the balance between NO $^{\bullet}$ production and EIF2AK activity could change the responses of cells to environmental stimuli. Therefore alteration of this balance could be a potential target for treating various diseases, such as artery plaque formations and cancers.

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A. 5-coordinate Heme-Fe(II)NO Complex

B. 6-coordinate Heme-Fe(III)HRI Complex

C. Proposed Mechanisms for reversing Hg^{2+} -inhibited HRI by $\text{NO}^\bullet$

Figure 1. Regulation of HRI activation by $\text{NO}^\bullet$

Panel A: The 5-coordinate Heme-Fe(II) $\text{NO}^\bullet$ Complex in active HRI. Panel B: The 6-coordinate Heme-Fe(III)HRI Complex in inactive HRI. Panel C: Mechanism proposed for Hg^{2+} -inhibition of HRI and the reverse reaction by $\text{NO}^\bullet$.

Figure 2. Mechanisms of NO[•]-induced activation of PERK

Panel A: Ca^{2+} depletion-mediated activation of PERK. Panel B: S-nitrosylation-mediated activation of PERK. Panel C: NO[•]-induced and UPR-mediated cell apoptosis pathways.

Figure 3.
The regulation of GCN2 activity by NOS under cell culture condition.

A: DsRNA-induced and PKR-mediated iNOS expression

B: PKR and iNOS-mediated cellular responses

Figure 4. Models for NO[•] production mediated by PKR and the cellular responses to activated PKR

Panel A: DsRNA-induced and PKR-mediated activation of iNOS. Panel B: The cellular response to activated PKR.

Table I

Standard Reduction Potentials in aqueous solution at 25 °C

Half reaction	E°(V)	Solution
$2\text{Hg}^{2+}(\text{aq}) + 2e^{-} \rightarrow \text{Hg}_2^{2+}(\text{aq})$	0.92	Acidic
$2\text{Hg}^{2+}(\text{aq}) + 2e^{-} \rightarrow 2\text{Hg}(\text{l})$	0.85	Acidic
$\text{Fe}^{3+}(\text{aq}) + e^{-} \rightarrow \text{Fe}^{2+}(\text{aq})$	0.77	Acidic
$\text{NO}_2^{-} + e^{-} + \text{H}_2\text{O} \rightarrow \text{NO}^{\bullet} + 2\text{OH}^{-}$	0.37	Neutral
$\text{Cu}^{2+}(\text{aq}) + e^{-} \rightarrow \text{Cu}^{+}(\text{aq})$	0.15	Acidic
$\text{Pb}^{2+}(\text{aq}) + 2e^{-} \rightarrow \text{Pb}(\text{s})$	-0.13	Acidic
$\text{Cd}^{2+}(\text{aq}) + 2e^{-} \rightarrow \text{Cd}(\text{s})$	-0.40	Acidic
$\text{Zn}^{2+}(\text{aq}) + 2e^{-} \rightarrow \text{Zn}(\text{s})$	-0.76	Acidic