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Old target new approach: an alternate NF-κB activation pathway via translation inhibition

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Abstract

Activation of the transcription factor NF-κB is a highly regulated multi-level process. The critical step during activation is the release from its inhibitor IκB, which as any other protein is under the direct influence of translation regulation. In this review, we summarize in detail the current understanding of the impact of translational regulation on NF-κB activation. We illustrate a newly developed mechanism of eIF2α kinase-mediated IκB depletion and subsequent NF-κB activation. We also show that the classical NF-κB activation pathways occur simultaneously with, and are complemented by, translational down regulation of the inhibitor molecule IκB, the importance of one or the other being shifted in accordance with the type and magnitude of the stressing agent or stimuli.

Keywords

Inhibitor of nuclear factor κB; Nuclear factor κB; Eukaryotic initiation factor 2; eIF2α kinase; IκB kinase

History

In 1986 David Baltimore's laboratory discovered a nuclear protein in mature B cells that binds to a 10 nucleotide stretch of double-stranded DNA in the κ immunoglobulin light chain enhancer (GGGACTTTCC) [1]. It was soon proven that this nuclear factor had a role in the mediated expression of the κ light chain and that it's localization in the nuclei is associated with different cellular stimuli [2]. Further studies have shown that NF-κB is involved in the regulation of the expressions of many genes that are mostly related to the immune and inflammatory response, along with genes determining developmental processes, cellular growth, and apoptosis [3,4].

NF-κB family members

The mammalian NF-κB family is composed of five members, i.e., p65 (RelA), RelB, NF-κB1 (p50 and its precursor p105), c-Rel, and NF-κB2 (p52 and its precursor p100) [5,6]. They all have in common a 300 amino acid Rel homology domain (RHD) located close to the N terminus of the protein [7]. However, while p65 and p50 were found to be universally present, the other three members (RelB, cRel, and p52) were suggested to be only expressed in lymphoid cells [8]. The RHD contains sequences are accountable for the homo- or hetero-dimerization of the

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family members. Of the five members, only three p65, RelB, and c-Rel contain a *trans*-activation domain (TAD), which is needed to promote transcription by facilitating the employment of activators and banishment of repressors [9]. Subsequently homodimers of the other two members, p52 and p50 are unable to activate transcription. Instead, they attenuate expression of target genes.

The role of IκB in regulation of NF-κB activation

The activity of NF-κB is regulated at multiple levels. The best known regulatory step is the cytoplasmic to nuclear transport of activated NF-κB p65:p50 heterodimer [10,11]. Without stimulation, cytoplasmic compartmentalization of NF-κB in cells is due to binding through the RHD to a member from the family of proteins called inhibitor of NF-κB (IκB). IκB family consists of IκBα, IκBβ, IκBε, IκBγ, BCL-3, and the two NF-κB precursors p100 and p105 [12,13]. IκBα and IκBβ achieve the cytoplasmic localization by masking the nuclear localization sequence (NLS) of amino acids on the NF-κB p65 subunit [14–16]. Failure to mask the NLS of the p65 subunit in addition to the existence of a nuclear export sequence (NES) on IκBα and p65, results in the constant shuttling of IκBα:p65:p50 complexes between the cytoplasm and nucleus. On the other hand, IκBβ:p65:p50 complexes are restricted to the extra nuclear compartment, this phenomena adding to the complexity of NF-κB regulation.

The role of kinases in regulation of NF-κB activation

After removing IκB , a second level of regulation is conferred mainly by stimulus-induced phosphorylation of NF-κB [17]. A protein kinase A (PKA) phosphorylation site was identified on both p65 and c-Rel at Ser 276, located 25 amino acids from the NLS, inside the Rel homology domain (RHD) [18]. Over-expression of PKA leads to a higher DNA-binding activity of NF-κB. This is mainly due to the fact that phosphorylated Ser 276 inhibits intermolecular association with inhibitors, thus facilitating nuclearization and DNA binding [17,19]. The same phosphorylation also promotes interaction with coactivator CREB binding protein (CBP/p300) [18]. A similar mechanism of NF-κB activation was identified during tumor necrosis factor α (TNFα) stimulation when p65 phosphorylation occurred at Ser 529 mediated by casein kinase II (CKII) [20,21]. Also during TNFα stimulation another activating phosphorylation occurs at Ser 536 by none other than IKK [22]. It is worthy to note that the same catalytic activity of IKK is required for IκB phosphorylation followed by ubiquitination and NF-κB activation by direct phosphorylation, fact that adds to the complexity of IKK mediated NF-κB activation [23]. The activity of stimulated NF-κB is down regulated by a feedback pathway through the newly synthesized IκBα, one of the first genes activated by NF-κB. The re-synthesized IκBα enters the nucleus, binds to NF-κB and exports it to the cytosol, thus inhibiting its functionality [24,25].

The classical NF-κB activation mechanism

Upon extra- or intracellular stimulation the IκBs are phosphorylated by an IκB kinase (IKK), ubiquitin targeted and undergo proteosomal degradation thus automatically exposing the NLS necessary for NF-κB nuclear localization [26,27]. IKK is a 700 kDa protein complex consisting of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ or NEMO—NF-κB essential modulator) [28–30]. Activation of the catalytic subunits takes place by phosphorylation followed by intra- and intermolecular *trans*-autophosphorylation releasing their kinase domains. A host of NF-κB inducers have been recognized so far, they consist of but are not limited to proinflamatory cytokines (TNFα, IL-1, etc.), double-stranded RNA (dsRNA), viruses and a variety of cell stressors like ultraviolet light (UV), reactive oxygen species (ROS), and genotoxic agents [7,31]. Some of NF-κB activators, such as cytokines, achieve activation through the classical activation mechanism (Fig. 1), while others like UV, ROS, heat shock, and hypoxia regulate NF-κB through much more branched and complex

cellular pathways [32–35]. The common feature of these general inducers is that they cause translation inhibition as a defense cellular response through their noxious effects.

The impact of translation initiation on NF-κB activation

An entirely different approach to NF-κB activation is provided by translational regulation via the eukaryotic initiation factor 2 (eIF2). During the initiation step of translation, eIF2 forms a complex with GTP and Met-tRNA forming a ternary complex, which associated with the small ribosomal unit contributes to the selection of the start codon. The release from the ribosome is achieved at the expense of hydrolization of GTP to GDP. In order to restart the initiation cycle the guanine exchange factor eIF2B refreshes the eIF2-GDP to eIF2-GTP [36]. The phosphorylation on Ser 51 of the α subunit of eIF2 (eIF2α) stabilizes the eIF2-GDP-eIF2B initiation complex preventing GDP-GTP exchange, thus halting the translational initiation process [37,38]. The eIF2α phosphorylation inhibits initiation of protein synthesis at a general level, allowing only the selective translation of some proteins that are required for mounting a stress response [39,40].

Key players in translational regulation are a host of serine–threonine kinases that can phosphorylate the Ser 51 of eIF2α. Four eIF2α kinases (EIF2AKs) have been identified. While each of the EIF2AKs has its own specific inducers, some stimulus such as UV and hypoxia also activate one or more of the kinases (Fig. 2).

EIF2AK1, known as the heme-regulated inhibitor kinase (HRI), is a critical component during erythroid maturation that regulates the stoichiometric ratio of hemoglobin components, i.e., α-globin, β-globin, and heme [41]. Two separate heme binding sites were identified in HRI [42]. HRI is activated by heme deficiency in multi-stages through series of *auto*-phosphorylations [43]. The phosphorylation of HRI first stabilizes its monomer that lacks eIF2α kinase activity, but has first heme-binding site occupied. Further phosphorylation of HRI induces the dimerization and confers heme sensitivity [44]. During high heme concentrations, heme binds to the second binding site inhibiting HRI kinase activity thus allowing for protein and implicitly hemoglobin translation [45]. In the situation of insufficient heme accumulation the second heme-binding site remains unoccupied, which leads to the induction of HRI kinase activity and inhibition of translation by eIF2α phosphorylation [46]. While heme deficiency leads to activation of NF-κB, there is no direct evidence yet to show that the translation inhibition is involved in the activation of signaling pathways. Besides heme deficiency, other NF-κB activator, such as arsenite-induced oxidative stress and heat shock were also found to activate HRI [47].

EIF2AK2, known as the interferon-induced double-stranded (ds) RNA-dependent protein kinase (PKR), plays a critical role in anti-viral defense [48]. The binding of the dsRNA exposes an ATP-binding site inducing dimerization and subsequent *auto*-phosphorylation leading to an active form of PKR [49–51].Avariety of stimuli, like growth factors and cytokines, activate PKR independently of dsRNA through PKR-associated activator proteins [52,53]. Initially, PKR was suggested to directly phosphorylate IκB [54]. However, the hypothesis was challenged by results showing that kinase inactivated PKR is still capable of activating NF-κB [55]. Furthermore, co-immunoprecipitation analysis demonstrated that PKR forms a complex with IKK independent of its ability of activation of NF-κB [55,56]. Based on these findings, it was proposed that PKR binds to the IKK complex or acts upstream facilitating IKK to phosphorylate IκBα at serines 32 and 36 [55–58]. Conversely, it has also been reported by others that PKR mutants that are unable to activate NF-κB still preserve their ability to co-immunoprecipitate with IKK [56]. While the roles of PKR and its catalytic activity in NF-κB activation remain controversial [55–58], several PKR activators, such as dsRNA and interferon γ (IFNγ) have been shown to induce NF-κB activation. Regardless of PKRs' inability to activate

NF-κB independently of its kinase function, activated PKR does nevertheless phosphorylate eIF2α thus inhibiting global translation and potentially can decrease IκB synthesis.

EIF2AK3, also known as the PKR like endoplasmic reticulum (ER) related kinase (PERK), is an ER membrane localized kinase [59–61]. Its inactive monomer state is stabilized by an ER chaperone immunoglobulin (Ig) heavy chain binding protein (BiP). Under ER-stress, BiP releases PERK, which undergoes dimerization, *trans*-phosphorylation and sequentially activation [60–63]. Outside or inside perturbations negatively affect protein-folding process in ER resulting in an accumulation of malfolded proteins, which triggers the unfolded protein response (UPR). While UPR transcriptionally activates the expression of ER chaperone to facilitate the folding process, it translationally inhibits general protein synthesis through phosphorylating eIF2α to reduce the accumulation of newly synthesized proteins in ER [64]. The converging point between the accumulation of unfolded proteins and global translation inhibition by eIF2α phosphorylation was determined to be PERK [59,65]. The PERK-mediated eIF2α phosphorylation and translation inhibition was shown to be directly involved in ER-stress-mediated NF-κB activation upon various stimuli, such as hypoxia, UV, and thapsigargin [33,34,66–69].

EIF2AK4 is also known as the amino acid starvation dependent general control of amino acid biosynthesis kinase (GCN2) [70,71]. It is an amino acid abundance controlled eIF2α kinase, which is activated during amino acid starvation. Its specific role is to halt protein translation while activating the translation of factors that are needed in amino acid synthesis [61,72]. The activation mechanism involves a histidyl-tRNA synthase (HisRS) homologous sequence, where the excess of uncharged tRNAs bind during amino acid deprivation [73]. A C-terminal RNA binding region is also required for its dimerization, activation, and association with ribosome [74,75]. The GCN2-mediated eIF2α phosphorylation and translation inhibition was shown to be directly involved in amino acid starvation induced NF-κB activation [66]. Besides nutritional stresses, the HisRS similar sequence also allows for activation by other stresses, such as UV and proteosome inhibition [71,72]. While there is no evidence yet to show that GCN2 is directly involved in NF-κB activation upon proteosome inhibition, it has been demonstrated that GCN2 mediates UV-induced NF-κB activation [76].

Besides eIF2α another initiation factor was recently also found to regulate NF-κB. This is the eukaryotic initiation factor 4E (eIF4E), which facilitates translation by binding to the 5′ cap structure of the mRNA. Although for now the studies fall short of providing any details for the activation mechanism, they offer other possible alternatives to the classical NF-κB activation pathway [77].

Regulation of IκB turnover

NF-κB is stranded in the cytoplasm bound by its inhibitor protein IκB. Even though both IκBα and IκBβ are able to inhibit NF-κB, it is IκBα that bears the major role in regulating its activation [78]. NF-κB is a transcription factor that has a fast response time in order to react promptly to cellular stress. In order to achieve this fast activation, the IκB levels are tightly and rapidly regulated [79]. While activation of receptor signaling cascade, such as TNFα and interleukin-1 (IL-1), often leads to phosphorylation, ubiquitination, and proteolysis of IκB , the more general cellular stimulus, such as UV and hypoxia, also possess the ability to induce translational inhibition of IκB synthesis.

IκB degradation occurs through two mechanisms, i.e., a signal-dependent and signal-independent (basal degradation) process [80]. IκB turnover is tightly linked to its structural domains. The centrally located ankyrin repeats are necessary for NF-κB binding and the two terminal regions are implicated in the degradation of IκB . The N-terminal sequence contains two IKK phosphorylation sites Ser 32 and 36 [81–84] and two ubiquitination sites Lys 21 and

22 [85,86]. The phosphorylation and ubiquitination of these sites promote IκB degradation in the 26S proteosome. The C-terminal region contains a PEST domain (Pro, Glu, Asp, Ser, and Thr rich regions), which is associated in general with high turnover proteins [87]. The PEST site in addition to multiple casein kinase II (CKII) phosphorylation sites on the C-terminal region are needed for both signal-induced degradation [84,88,89] and basal turnover of IκB [17,82,90,91].

The rate of degradation of IκB is also very much influenced by its association with NF-κB. Free IκB has a 30–40 min half-life, but the NF-κB associated one has a fivefold longer degradation time [92–95]. Free IκB constitutes only a 15% fraction of the total cellular IκB [92], and is a weak substrate for IKK phosphorylation [96]. The basal turnover of free IκB requires the CKII phosphorylation sites while the signal-dependent degradation is induced by IKK phosphorylation. For the NF-κB associated IκB , the basal turnover is also regulated by CKII phosphorylation, while the signal-induced degradation is regulated by both CKII and IKK phosphorylation [80,95,97–99] (Table 1).

While IKK and CKII regulate the removal rate of IκB , the EIF2AKs determine the synthetic rate of IκB . The phosphorylation of eIF2α by EIF2AK leads to the inhibition of global protein synthesis, including IκB. Since IκB has a relatively high basal turnover rate [79], the inhibition of new IκB synthesis results in a rapid depletion of IκB thus shifting the dynamic balance from NF-κB associated IκB toward free NF-κB and IκB (Fig. 3).

Targeting NF-κB for therapeutic development

NF-κB plays an important role in regulation of the process of innate and adaptive immune responses. Its ability to activate transcription of genes encoding cytokines (e.g., TNFα, IL-1, IL-2, and IL-6), chemokines, adhesion molecules (e.g., ICAM, VCAM, and E-selectin), inducible enzymes (e.g., iNOS and COX-2), and antimicrobial peptides (β defensine) gives it a central role in the overall process of immune response [78]. NF-κB also regulates genes outside the immune system presumably having an anti-apoptotic effect that would give an opportunity to the cell to repair DNA damage. Deregulation of these genes may lead to many diseases, such as cancer, atherosclerosis, arthritis, AIDS, etc. [3]. NF-κB has been a target for the development of therapeutics for many diseases [100]. Since eIF2α phosphorylation also impacts NF-κB activation, compounds that affect eIF2α phosphorylation through the aforementioned kinases will be potential therapeutics for treatment of various diseases. Indeed, several ER-stress inducing drugs are already in the spotlight for their ability to induce apoptosis in malignant cells. The chemotherapeutic agents doxorubicin and cisplatin, although known to mainly target DNA, were also shown to induce ER-stress and activate PERK [101–104]. Interferon and TNF-α are both antiviral proteins that have been used in combination with chemo- and radiation therapy and that possess the ability to activate PKR [105,106]. In addition, the potential for successful use of proteasome inhibitors for cancer treatment may be granted by the ability of these compounds to induce apoptosis through the blocking of protein degradation, which implicitly leads to ER-stress. The anti-multiple myeloma drug Velcade (PS-341), for example, which is a proteasome inhibitor, inhibits IκB degradation. In fact, Velcade was also shown to disrupt protein folding in the ER resulting in ER-stress [107–109]. In summary, elucidating the role of EIF2AK in mediation of NF-κB activation may lead us to a better understanding of the mechanisms of current NF-κB targeting drugs and development of new therapeutics to treat diseases related to deregulation of NF-κB.

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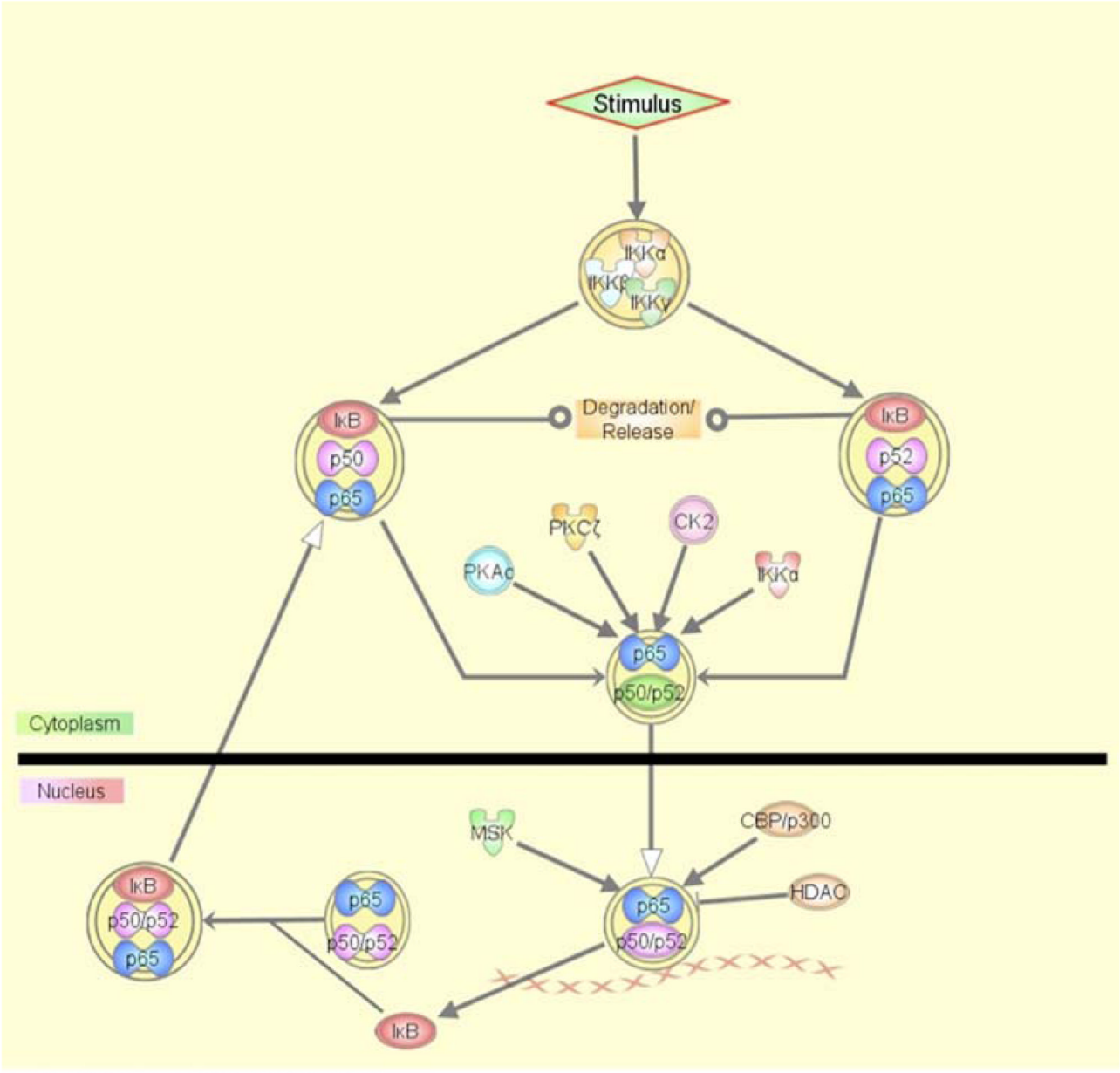

Fig. 1.

The classical NF-κB activation pathway. Stimulus induced IKK phosphorylates IκB inducing its degradation. Free NF-κB translocates to the nucleus and binds to the target DNA, while its transactivation efficiency and ability to recruit other activators is further regulated by different kinases. IκB provides feedback inhibition through expulsion of NF-κB from the nucleus. The graphical representation of the molecular network was generated through the use of Ingenuity Pathways Analysis (Ingenuity® Systems). All lines are supported by at least one reference from the literature or from information of canonical pathways stored in the Ingenuity Pathways Knowledge Base

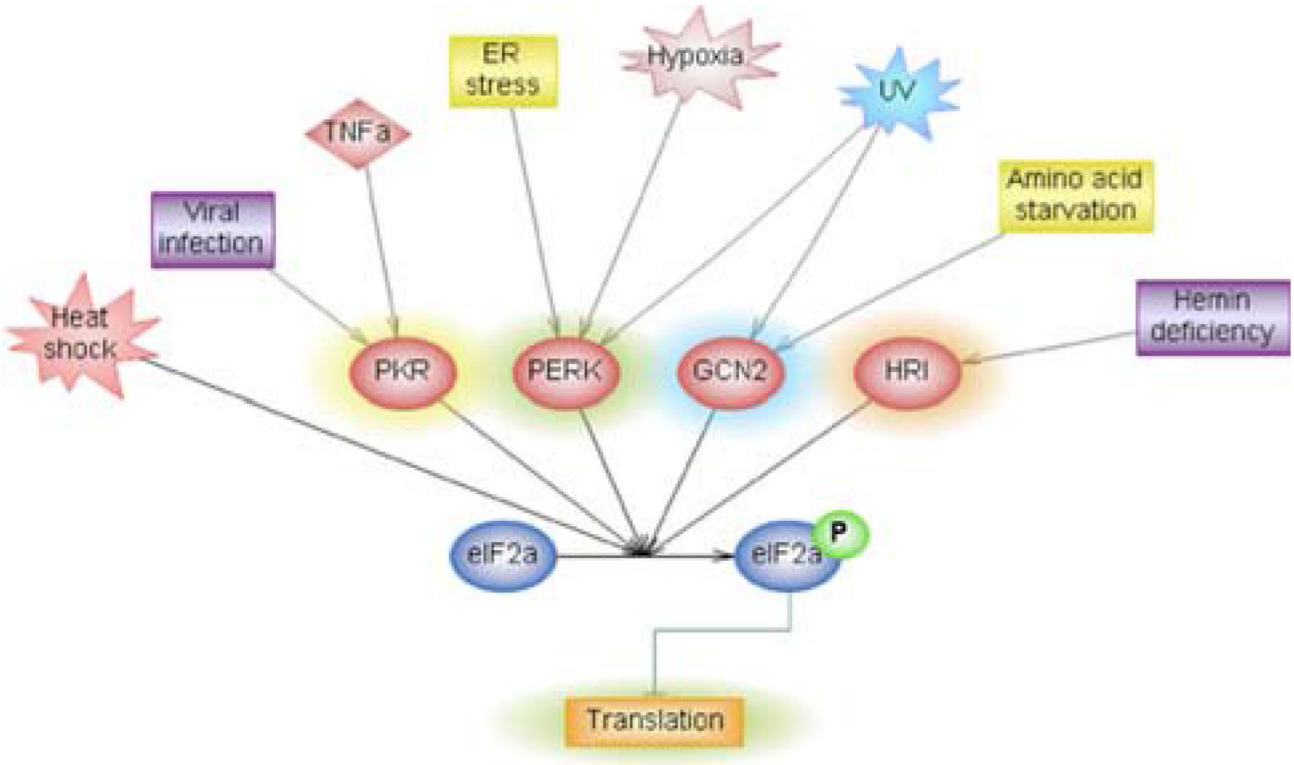

Fig. 2.

The eIF2 kinase regulated signaling pathways. A number of stimuli achieve eIF2α phosphorylation through the four known eIF2α kinases. The graphical representation of the molecular network was generated through the use of Pathway Studio 6 (Ariadne Genomics®). All lines are supported by at least one reference from previously published literature stored in the Pathway Studio database

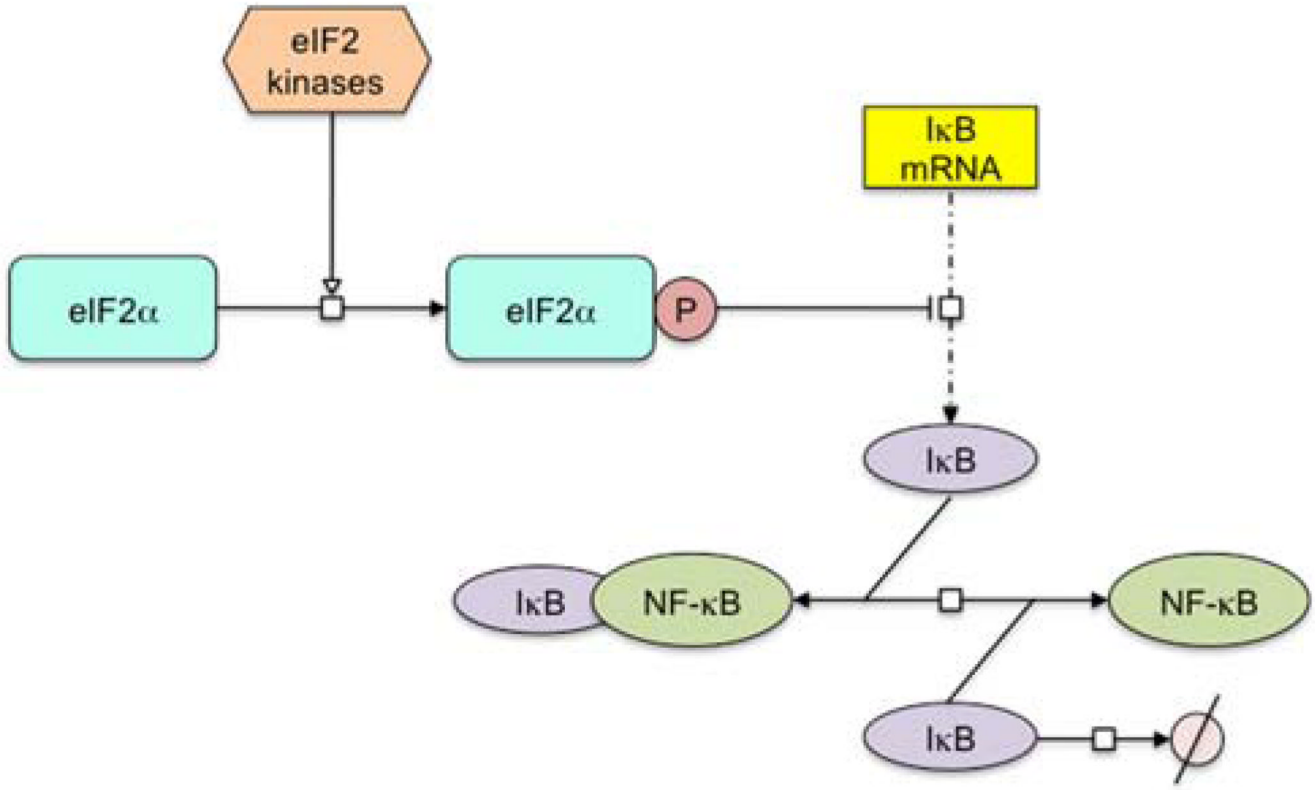

Fig. 3.
Model for translation regulation of NF-κB activation. The eIF2 kinases phosphorylate the α subunit of eIF2, which results in the translation inhibition of IκB synthesis. The reduction of IκB leads to the dissociation of IκB-NF-κB complex and subsequent NF-κB activation. The molecular network was depicted with CellDesigner® diagram editor

Table 1

Role of kinases in regulation of IκB turnover

IκB state	Basal turnover	Signal-induced degradation
Free	CKII	IKK
NF-κB associated	CKII	IKK + CKII