## Phylogeny

EIF2AK2 (PKR) is a member of the eIF2α kinase family, a group that also includes HRI, PERK, and GCN2 (dabo2012dsrnadependentproteinkinase pages 1-3, dar2005higherordersubstraterecognition pages 1-3, unknownauthors2006catalyticswitchingand pages 42-46). This family phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α) (bounader2019thesearchfor pages 1-3, unknownauthors2006catalyticswitchingand pages 42-46). According to the kinome classification by Manning et al. 2002, EIF2AK2 is assigned to the CMGC group of kinases (dar2005higherordersubstraterecognition pages 12-13, donnelly2013theeif2αkinases pages 4-5, dabo2012dsrnadependentproteinkinase pages 6-8). EIF2AK2 is a vertebrate-specific kinase that is suggested to have evolved from gene duplication of ancestral eIF2α kinases such as GCN2 and HRI (rothenburg2016evolutionofeif2α pages 4-7). Phylogenetic analysis shows that the C-lobe of the EIF2AK2 kinase domain is homologous to the baculovirus protein PK2, which suggests a horizontal gene transfer event from an insect host (li2015baculovirusproteinpk2 pages 3-3).

## Reaction Catalyzed

EIF2AK2 catalyzes the ATP-dependent transfer of the γ-phosphate group to a serine or threonine residue on a protein substrate (dar2005higherordersubstraterecognition pages 14-14, donnelly2013theeif2αkinases pages 4-5). The general chemical reaction is summarized by the formula: ATP + [protein] → ADP + [phosphoprotein] (dey2014activationofprotein pages 2-3, dar2005higherordersubstraterecognition pages 14-14, cesaro2021pkractivitymodulation pages 9-10). Its most characterized reaction is the phosphorylation of its primary substrate, the eukaryotic initiation factor 2 alpha subunit (eIF2α), which can be expressed as: ATP + eIF2α → ADP + phosphorylated eIF2α (barber2005thedsrnadependentprotein pages 2-3, dey2014activationofprotein pages 2-2).

## Cofactor Requirements

The kinase activity of EIF2AK2 is dependent on the presence of divalent metal ions, specifically Mg²⁺, which acts as an essential cofactor (dar2005higherordersubstraterecognition pages 12-13, donnelly2013theeif2αkinases pages 4-5, dey2014activationofprotein pages 11-12). Mg²⁺ is required to stabilize ATP binding and facilitate the phosphotransfer reaction (li2015baculovirusproteinpk2 pages 2-2, bounader2019thesearchfor pages 1-3).

## Substrate Specificity

The primary substrate of EIF2AK2 is the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α), which it phosphorylates at Serine 51 (dar2005higherordersubstraterecognition pages 1-3, bounader2019thesearchfor pages 1-3). Substrate recognition is complex, requiring higher-order interactions beyond the phosphorylation site, as peptides containing only the Ser51 motif are poor substrates (dar2005higherordersubstraterecognition pages 1-3). Efficient phosphorylation requires the full-length eIF2α protein, which interacts with an extended docking surface on the kinase involving the αG-helix (taylor2005pkrandeif2α pages 1-2). A positional scanning peptide library approach has defined the consensus substrate motif for EIF2AK2, which shows preferences for specific amino acids at positions -5 to +5 relative to the phosphorylation site (johnson2023anatlasof pages 2-3). EIF2AK2 is primarily a serine/threonine kinase, but it also exhibits dual specificity, with the ability to phosphorylate tyrosine residues (cesaro2021pkractivitymodulation pages 1-2, unknownauthors2006catalyticswitchingand pages 119-122).

## Structure

EIF2AK2 is a 551 amino acid protein with a modular structure comprising an N-terminal regulatory region and a C-terminal catalytic kinase domain (KD) (dabo2012dsrnadependentproteinkinase pages 1-3). The N-terminal domain contains two tandem double-stranded RNA-binding motifs (dsRBMs), which recognize and bind dsRNA (bounader2019thesearchfor pages 1-3, unknownauthors2006catalyticswitchingand pages 46-51). The KD (residues 258-551) possesses the conserved bilobal architecture of protein kinases, with a smaller N-lobe and a larger C-lobe (bounader2019thesearchfor pages 1-3, unknownauthors2006catalyticswitchingand pages 114-119). EIF2AK2 forms a homodimer through a back-to-back interaction mediated by the N-lobes of the KD (unknownauthors2006catalyticswitchingand pages 114-119, dey2014activationofprotein pages 7-8). The C-lobe contains the ATP-binding site and the primary docking site for the eIF2α substrate, which involves a unique αG-helix (bounader2019thesearchfor pages 1-3, taylor2005pkrandeif2α pages 1-2). Key regulatory elements include the activation segment (residues 432-458), which contains the critical autophosphorylation site Thr446, and the C-helix (αC), which coordinates with the phosphorylated activation loop to stabilize the active conformation (unknownauthors2006catalyticswitchingand pages 119-122, dey2014activationofprotein pages 2-3).

## Regulation

Activation of EIF2AK2 is initiated by binding to dsRNA molecules longer than 30-35 base pairs, which promotes homodimerization and subsequent cis-autophosphorylation (rothenburg2016evolutionofeif2α pages 14-16, unknownauthors2008theactivationand pages 28-34, dey2014activationofprotein pages 1-2). Autophosphorylation on multiple serine/threonine residues, critically at Thr446 and Thr451 in the activation loop, is required for full catalytic activity (barber2005thedsrnadependentprotein pages 2-3, dabo2012dsrnadependentproteinkinase pages 1-3). Other post-translational modifications (PTMs) modulate its function; ISGylation and SUMOylation at lysines K60, K69, K150, K159, and K440 cause constitutive activation (bounader2019thesearchfor pages 12-15). Conversely, phosphorylation at Ser6 and Ser97, located near the dsRBMs, acts as a negative feedback mechanism (cesaro2021pkractivitymodulation pages 9-10, cesaro2021pkractivitymodulation pages 1-2). EIF2AK2 activity is also regulated by protein-protein interactions. The cellular protein PACT (also known as RAX) is an activator, while TRBP, ADAR1, and hDus2 act as inhibitors (barber2005thedsrnadependentprotein pages 2-3, bounader2019thesearchfor pages 12-15). NF90 can function as both an activator and an inhibitor (bounader2019thesearchfor pages 12-15).

## Function

EIF2AK2 is an interferon-inducible kinase located in the cytosol and nucleus that is a central mediator of the innate immune response to viral infection and the integrated stress response (ISR) (bounader2019thesearchfor pages 1-3, donnelly2013theeif2αkinases pages 4-5). Upstream signals that trigger its activation include viral dsRNA, oxidative and ER stress, cytokines, and the protein activator PACT (barber2005thedsrnadependentprotein pages 2-3, donnelly2013theeif2αkinases pages 4-5). The principal downstream function of activated EIF2AK2 is the phosphorylation of its substrate, eIF2α (encoded by EIF2S1), on Serine 51 (barber2005thedsrnadependentprotein pages 2-3). This phosphorylation converts eIF2α into a competitive inhibitor of its guanine nucleotide exchange factor, eIF2B, which leads to the attenuation of global protein synthesis, the formation of stress granules, and the promotion of apoptosis, thereby restricting viral replication (barber2005thedsrnadependentprotein pages 2-3, bounader2019thesearchfor pages 1-3, dabo2012dsrnadependentproteinkinase pages 6-8). EIF2AK2 also phosphorylates other substrates, such as p53, and functions as an adapter protein in signaling pathways including NF-κB, p38MAPK, and STAT (dabo2012dsrnadependentproteinkinase pages 1-3, donnelly2013theeif2αkinases pages 4-5).

## Inhibitors

EIF2AK2 is targeted by various viral and host inhibitors. Viral inhibitors include structured RNAs, such as adenovirus VA RNAi and Epstein-Barr virus EBER1/2, which bind to EIF2AK2 and prevent its activation (rothenburg2016evolutionofeif2α pages 14-16). Viral proteins also antagonize EIF2AK2; for example, the influenza virus NS1 protein inhibits it, the vaccinia virus E3L protein sequesters activator dsRNA, and the vaccinia virus K3L protein acts as a competitive inhibitor by mimicking the eIF2α substrate (bounader2019thesearchfor pages 12-15, rothenburg2016evolutionofeif2α pages 14-16, dar2005higherordersubstraterecognition pages 1-3). Cellular protein inhibitors include TRBP, hDus2, and ADAR1, which can suppress EIF2AK2 activity through direct interaction or by editing endogenous dsRNA activators (bounader2019thesearchfor pages 12-15).

## Other Comments

Dysregulation of EIF2AK2 is linked to several human diseases, including metabolic disorders, cancer, and neurodegenerative conditions such as Alzheimer’s disease (bounader2019thesearchfor pages 1-3). In the context of Alzheimer’s, phosphorylated EIF2AK2 has been observed to colocalize with pathological protein aggregates (dabo2012dsrnadependentproteinkinase pages 6-8). A number of disease-associated and experimentally characterized mutations affect its function. The K296R mutation within the catalytic domain is a well-characterized loss-of-function mutation that abolishes kinase activity (dabo2012dsrnadependentproteinkinase pages 1-3, dey2014activationofprotein pages 7-8). Mutations that disrupt the kinase domain dimerization interface also impair EIF2AK2 activation (bounader2019thesearchfor pages 1-3). Additionally, phosphomimetic mutations at Ser6 and Ser97 have been shown to inhibit kinase activation, demonstrating the regulatory role of these residues (cesaro2021pkractivitymodulation pages 1-2).

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