1. Phylogeny  
   Eukaryotic elongation factor 2 kinase (EEF2K), UniProt ID O00418, is an atypical serine/threonine kinase that diverges significantly from conventional eukaryotic protein kinases. It is classified into the alpha‐kinase family, a distinct evolutionary branch characterized by a unique catalytic domain that does not share substantial sequence similarity with classical eukaryotic protein kinases. Comparative phylogenetic analyses reveal that EEF2K orthologs are conserved across a broad spectrum of eukaryotic species, including vertebrates and several invertebrates, although reports indicate its absence in certain phyla such as insects and fungi. This suggests that EEF2K belongs to a core set of kinases that emerged early in eukaryotic evolution, with its evolutionary trajectory marked by a conserved catalytic motif and regulatory features that distinguish it from the conventional kinase families described in classical kinome studies (miranda‐saavedra2007classificationandfunctional pages 4-6, middelbeek2010thealphakinasefamily pages 1-2). In addition, the alpha‐kinase family, including EEF2K, is phylogenetically linked to other atypical kinases such as the Dictyostelium myosin heavy chain kinases and the TRPM6/7 channel kinases, underscoring not only its evolutionary conservation but also its functional divergence from the AGC, CAMK, and other conventional kinase groups (middelbeek2010thealphakinasefamily pages 2-3, piserchio2024revealingeef2kinase pages 14-17).
2. Reaction Catalyzed  
   EEF2K catalyzes the transfer of a phosphate group from ATP to its exclusive substrate, eukaryotic elongation factor 2 (eEF2). The chemical reaction can be summarized as:  
     ATP + eEF2 → ADP + eEF2-(Thr56)-phosphate + H⁺  
   This phosphorylation event occurs specifically on a threonine residue (Thr56) within eEF2, leading to a conformational change that inactivates eEF2’s ability to bind to ribosomes, thereby reducing the rate of peptide chain elongation during protein synthesis (kaul2011eukaryoticelongationfactor‐2 pages 2-3, ryazanov2002elongationfactor‐2kinase pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of EEF2K is dependent upon several cofactors. Primarily, the enzyme requires divalent magnesium ions (Mg²⁺) which are essential for ATP binding and proper orientation of the phosphate group for transfer. In addition, EEF2K activation is strictly dependent on calcium ions (Ca²⁺) in complex with calmodulin (CaM). The binding of Ca²⁺ to calmodulin induces conformational changes in both calmodulin and EEF2K, which is required for relieving autoinhibition and aligning the catalytic machinery for efficient phosphoryl transfer (kaul2011eukaryoticelongationfactor‐2 pages 2-3, lee2016structuralbasisfor pages 14-15).
4. Substrate Specificity  
   EEF2K exhibits an exceptionally high degree of substrate specificity, as its sole known physiological substrate is eukaryotic elongation factor 2 (eEF2). The enzyme phosphorylates eEF2 specifically at threonine residue 56, an event that effectively inactivates eEF2 by impairing its ability to engage with the ribosome. No additional consensus phosphorylation motif has been definitively characterized for EEF2K beyond this substrate‐directed specificity. The unique recognition and binding between EEF2K and eEF2 are mediated by specific docking interactions between the catalytic domain of the kinase and a corresponding substrate binding region on eEF2, ensuring that phosphorylation is confined exclusively to its target under physiological conditions (hooper2015structuralstudieson pages 52-56, kaul2011eukaryoticelongationfactor‐2 pages 2-3).
5. Structure  
   The structural organization of EEF2K is defined by an atypical domain architecture that deviates markedly from that of conventional protein kinases. It comprises an N-terminal calmodulin-targeting motif (CTM) that adopts an α-helical conformation necessary for robust binding to the hydrophobic face of calmodulin’s C-terminal lobe. Directly following the CTM is a regulatory element (RE) that, in conjunction with the calmodulin-bound CTM, contributes to the formation of an “activation spine” (A-spine). This spine is anchored by invariant tryptophan residues—specifically, W85 within the CTM and W99 in the regulatory element—which are essential for optimal catalytic activity (piserchio2024revealingeef2kinase pages 4-6). The central catalytic domain of EEF2K, classified as an alpha-kinase domain, is arranged into a dual-lobed structure; the N-terminal lobe is predominantly composed of β-sheets, while the C-terminal lobe is enriched in α-helices. This catalytic core, although functionally analogous to conventional kinase domains, exhibits distinct structural features such as a repositioned glycine-rich loop and a modified configuration of the ATP-binding site. Furthermore, the enzyme contains a long regulatory loop that harbors several autophosphorylation sites essential for full activation, and its C-terminal region includes Sel1-like repeats that may participate in protein–protein interactions and substrate binding (piserchio2024revealingeef2kinase pages 11-13, kaul2011eukaryoticelongationfactor‐2 pages 8-8). Although full-length high-resolution structures of EEF2K remain uncharacterized, minimal functional constructs (e.g., eEF-2KTR) in complex with calmodulin have provided significant insights into the conformational dynamics and molecular determinants of its activation (lee2016structuralbasisfor pages 8-9).
6. Regulation  
   EEF2K is regulated by a complex interplay of calcium signals, calmodulin binding, autophosphorylation events, and upstream kinase activities. Its catalytic activation is initiated when elevated intracellular calcium levels result in Ca²⁺ binding to calmodulin. Calmodulin, upon activation, engages the N-terminal calmodulin-targeting motif (CTM) of EEF2K, a binding event that induces a substantial conformational rearrangement. This rearrangement relieves autoinhibition and promotes alignment of the activation spine, which is further stabilized by the autophosphorylation of critical residues. One such key autophosphorylation site is Thr348; phosphorylation at this residue is required to achieve maximal kinase activation. In addition, phosphorylation at other sites, notably by kinases such as AMP-activated protein kinase (AMPK) and protein kinase A (PKA), further modulates EEF2K’s activity. For example, phosphorylation at a conserved serine residue (Ser500 in humans) facilitates Ca²⁺/calmodulin-independent activity, suggesting a mechanism through which EEF2K can sustain partial activity under conditions when calcium levels are suboptimal (kaul2011eukaryoticelongationfactor‐2 pages 7-8, browne2004anovelmtorregulated pages 10-11). Moreover, environmental factors such as mild intracellular acidification, which may occur during hypoxic stress or ischemia, have been shown to enhance EEF2K activation by increasing its affinity for calmodulin (hooper2015structuralstudieson pages 56-60). An additional layer of regulation is provided by allosteric binding of nucleotides such as ADP at a basic pocket located at the interface between calmodulin and the kinase domain; this event acts to lower the calmodulin concentration required for kinase activation, thereby linking the enzyme’s activity to cellular energy status (piserchio2024revealingeef2kinase pages 4-6, kaul2011eukaryoticelongationfactor‐2 pages 8-8).
7. Function  
   EEF2K plays a central role in the regulation of protein synthesis by modulating the elongation phase of translation. Upon activation, EEF2K phosphorylates eEF2 at threonine 56; this post-translational modification inhibits eEF2’s ability to mediate the translocation step of ribosomal movement along mRNA, resulting in a marked decrease in peptide chain elongation. This regulation of protein synthesis is crucial under conditions of cellular stress such as nutrient deprivation, hypoxia, or metabolic stress, where energy conservation is paramount. In these situations, EEF2K-mediated reduction in protein synthesis helps to redirect cellular resources towards stress adaptation and survival pathways. Moreover, EEF2K is intricately linked with key signaling pathways responsible for cellular energy homeostasis, including the AMPK and mTOR pathways. Upstream kinases such as AMPK activate EEF2K during cellular energy depletion, thereby reducing overall protein synthesis to conserve ATP, while insulin-mediated mTOR signaling converges onto EEF2K to suppress its activity in favor of anabolic processes under nutrient-replete conditions (kaul2011eukaryoticelongationfactor‐2 pages 3-4, ryazanov2002elongationfactor‐2kinase pages 1-2). Additionally, EEF2K activity is implicated in various physiological processes including synaptic plasticity, learning, and memory, where local modulation of translation rates is critical for neuronal function. In cancer cells, elevated EEF2K activity has been associated with enhanced survival under metabolic stress, which contributes to tumor progression and resistance to chemotherapy (wang2017eukaryoticelongationfactor pages 1-3, ejiri2002moonlightingfunctionsof pages 5-8).
8. Other Comments  
   Given its unique structure and central role in the regulation of translational elongation, EEF2K is an attractive target for therapeutic intervention, particularly in the context of cancer and certain neurodegenerative disorders. Several experimental inhibitors have been developed to modulate EEF2K activity; these inhibitors are designed to either disrupt calmodulin binding or directly impede the kinase’s catalytic activity. Although detailed inhibitor profiles and specificity data are still emerging, preclinical studies have demonstrated that pharmacological inhibition of EEF2K can sensitize tumor cells to chemotherapy and may reduce cancer cell proliferation. In addition, dysregulation of EEF2K activity—whether through aberrant autophosphorylation or misregulated upstream signaling—has been linked to pathological states, including ischemic injury in cardiac and cerebral tissues as well as in neurodegenerative processes. Although specific disease-associated mutations have not been extensively characterized, the pivotal role of EEF2K in integrating nutrient and stress signals underscores its potential as a biomarker for disease progression and as a candidate for targeted drug development. Furthermore, advances in structural studies using minimal functional constructs in complex with calmodulin continue to shed light on the molecular determinants of its regulation, thereby facilitating the rational design of highly selective inhibitors (huang2021kinorthoamethod pages 1-2, knebel2001anovelmethod pages 3-5).
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