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Eukaryotic elongation factor 2 kinase, an unusual enzyme with multiple roles

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A B S T R A C T

Eukaryotic elongation factor 2 kinase (eEF2K) is a member of the small group of atypical 'α-kinases'. It phosphorylates and inhibits eukaryotic elongation factor 2, to slow down the elongation stage of protein synthesis, which normally consumes a great deal of energy and amino acids. The activity of eEF2K is normally dependent on calcium ions and calmodulin. eEF2K is also regulated by a plethora of other inputs, including inhibition by signalling downstream of anabolic signalling pathways such as the mammalian target of rapamycin complex 1. Recent data show that eEF2K helps to protect cancer cells against nutrient starvation and is also cytoprotective in other settings, including hypoxia. Growing evidence points to roles for eEF2K in neurological processes such as learning and memory and perhaps in depression.

Introduction

The proper regulation of protein synthesis (mRNA translation) is crucial for cell function. Protein synthesis places heavy demands upon the cell in terms of its requirements for energy (ATP and GTP; the equivalent of at least 4 ATPs is used for every amino acid incorporated into a new protein) and

Abbreviations: 2-DOG, 2-deoxyglucose; AMPK, AMP-activated protein kinase; βTrCP, β-transducin repeat-containing protein; BDNF, brain-derived neurotrophic factor; CaM, calmodulin; eEF2, eukaryotic elongation factor 2; eEF2K, eEF2 kinase; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MHCK, myosin heavy chain kinase; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; NMDAR, N-methyl-D-aspartate receptor; S6K1, ribosomal protein S6 kinase 1; TNFα, tumour necrosis factor-α.

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amino acids, so protein synthesis is a key component of the cellular economy. Furthermore, recent proteome- and transcriptome-wide analyses have shown that protein synthesis plays the primary role (greater than those of, e.g., rates of transcription or protein degradation) in determining the cellular proteome (Schwanhausser et al., 2011). This reflects the fact that different mRNAs are translated with widely differing efficiencies.

Protein synthesis is conventionally divided into three main stages, initiation, elongation and termination (Merrick, 2010), although the recycling of ribosomes after termination may be considered a fourth stage. In terms of understanding the control of protein synthesis, most attention has been devoted to the initiation process, where ribosomes bind to the mRNA and locate the start codon; it is therefore evident that control of initiation can provide mechanisms for regulating the translation of specific mRNAs. However, there is growing evidence that control of elongation also plays a role in modulating the translation of specific mRNAs, and that it is important for cellular responses to lack of nutrients, energy and oxygen (which is required for efficient ATP production in many cells). This review will focus on recent developments in understanding the regulation by phosphorylation of eukaryotic elongation factor 2 (eEF2), the protein which mediates the translocation step of peptide-chain elongation during protein synthesis (i.e., the movement of the ribosome along the mRNA from one codon to the next).

eEF2K is an atypical 'α-kinase'

Eukaryotic elongation factor 2 kinase (eEF2K) belongs to a small group of atypical protein kinases, termed 'α-kinases', of which there are six members in the human genome (Ryazanov et al., 1999; Middelbeek et al., 2010). α-kinases show no sequence similarity to the main protein kinase superfamily, although they do display limited three-dimensional structural similarity (Yamaguchi et al., 2001; Ye et al., 2010). eEF2K is the only α-kinase whose activity is dependent upon Ca^{2+}-ions ((Ryazanov et al., 1988; Nairn et al., 1985); it was originally called 'Ca/CaM-kinase III'). The only known substrate for eEF2K is elongation factor eEF2. Very little is known about the regulation of the activity of the other five family members, or their substrates. Since phosphorylation of eEF2 at Thr56 (Price et al., 1991) impairs its binding to the ribosome (Carlberg et al., 1990), eEF2K acts to inhibit eEF2 and thus slow down the rate of elongation. This residue, and adjacent sequences, are exceptionally highly conserved, being identical even in budding yeast. However, eEF2K homologues are absent from many eukaryotes, e.g., fungi, plants and arthropods. Homologues of eEF2 found in nematodes and yeast can be phosphorylated on the equivalent of Thr56 by a different kinase, Rck2 (Teige et al., 2001).

Activation of eEF2K by Ca^{2+}-ions is conferred by calmodulin (CaM), which binds up to four Ca^{2+}-ions and interacts with a region in eEF2K close to, and on the N-terminal side of, its catalytic domain (Fig. 1). The mechanism by which Ca^{2+}/CaM activates eEF2K is unknown; in some other Ca/CaM-kinases, activation occurs due to the removal of an autoinhibitory helical feature from the active site (Rellos et al., 2010). However, it is not clear whether eEF2K contains a regulatory motif of this kind.

The overall layout of eEF2K is depicted in Fig. 1. Removal of a region of approximately 80 residues N-terminal of the CaM-binding motif enhances eEF2K activity (Pigott et al., 2011), suggesting it might play a regulatory role. The catalytic domain comprises approximately residues 125–320 in human eEF2K. C-terminal to this is an autophosphorylation site (Thr348) which is required for activity. In myosin heavy chain kinase A (MHCK A) from *Dictyostelium discoideum*, another α-kinase, the corresponding residue (also an autophosphorylated threonine) apparently docks into a 'phosphate-binding pocket' to help create the active conformation (Crawley et al., 2011). Sequence alignments suggest a similar mechanism may apply to eEF2K. eEF2K also undergoes autophosphorylation at additional sites, including Ser445 and, in one report, Ser500 (Pyr Dit Ruys et al., 2012; Tavares et al., 2012). The roles of these sites in the control of eEF2K is less clear than for Thr348, although phosphorylation of Ser500 decreases the dependence of eEF2K on Ca^{2+}-ions. Autophosphorylation occurs in an intramolecular manner (Redpath and Proud, 1993a,b).

Beyond the catalytic domain is a region which is predicted to be unstructured but contains several phosphorylation sites that regulate eEF2K activity (Fig. 1; see also below). This is followed by four predicted α-helical 'SEL1-like' motifs, a feature thought to be involved in protein:protein interactions (Mittl and Schneider-Brachert, 2007) although the partners for these motifs in eEF2K remain to be