Regulation of cap-dependent translation by eIF4E inhibitory proteins

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Eukaryotic messenger RNAs contain a modified guanosine, termed a cap, at their 5′ ends. Translation of mRNAs requires the binding of an initiation factor, eIF4E, to the cap structure. Here, we describe a family of proteins that through a shared sequence regulate cap-dependent translation. The biological importance of this translational regulation is immense, and affects such processes as cell growth, development, oncogenic transformation and perhaps even axon pathfinding and memory consolidation.

A single cell never exploits the full panoply of gene products available for its use; at any one time, the transcription of most genes is unwarranted and therefore these genes are silenced. Even for those mRNAs that are synthesized and transported to the cytoplasm, however, there are often other levels of regulation.

The past few years have witnessed an explosion in the number of mRNAs whose translation is recognized to be temporally and spatially regulated in various cell types. Although no single mechanism controls the translation of all mRNAs, emerging evidence indicates that the regulated binding of translation initiation factors (eIFs) to the 7-methyl guanosine residue that caps the 5′ ends of all nuclear-encoded eukaryotic mRNAs is important. In particular, the interaction of the ribosomal-subunit-associated eIF4G with the cap-bound eIF4E is necessary for cap-dependent translation. A group of factors generically known as eIF4E inhibitory proteins modulate the eIF4G–eIF4E interaction. Whereas some eIF4E inhibitory proteins repress translation by associating with eIF4E on a large number of transcripts, others are tethered to specific subsets of mRNAs through interactions with RNA binding proteins, thus restricting their inhibition of translation to only certain mRNAs. Biologically, the eIF4E inhibitory proteins are enormously important; they control development and cell growth, repress tumour formation, and may influence critical neuronal events such as axon guidance and synaptic plasticity, a phenomenon that may underlie long-term memory storage.

Here, we present not only the molecular mechanisms by which some of these proteins control translation, but also describe a few of the biological processes they regulate. Although only a handful of these eIF4E inhibitory proteins have been identified, we suspect that there may be several others that await discovery.

The translation initiation machinery

Initiation is the rate-limiting step in translation and is the most common target of translational control. The mRNA 5′ cap is bound by eIF4F, a heterotrimeric protein complex that is the focal point for initiation. eIF4G is the backbone of this complex; it interacts not only with eIF4E, but also with eIF4A, an RNA helicase that facilitates ribosome binding and its passage along the 5′ untranslated region (UTR) towards the initiation codon. eIF4G also associates with eIF3, a multisubunit factor that bridges the proteins bound to the mRNA's 5′ end with the 40S ribosomal subunit (Fig. 1). This ribosomal subunit comes 'pre-charged' as a ternary complex composed of eIF2, GTP and the initiator methionine-transfer RNA. With the aid of eIF4 initiation factor as well as ATP, this agglomeration of RNA and protein is thought to scan the mRNA in the 5′ to 3′ direction. When it encounters an AUG start codon in an optimal context, other factors as well as the 60S ribosomal subunit are recruited and polypeptide chain elongation begins[1].

The eIF4E–eIF4G interface is an important target for translational control. The core portion of eIF4G that interacts with eIF4E is small—about 15 amino-acid residues[2]. Strikingly, several other proteins contain similar peptide motifs, and it is this region that competes with eIF4G for binding to eIF4E; in this manner they control the rate of 40S ribosomal subunit association with mRNA, and hence translation initiation. A clear demonstration of why the competition between eIF4G and other proteins for interaction with eIF4E is so effective in preventing translation comes from X-ray crystallographic analysis. Peptides derived from the regions of eIF4G and an eIF4E inhibitory protein called 4E-BP (for 4E-binding proteins, also known as PHAS-I for phosphorylated heat and acid

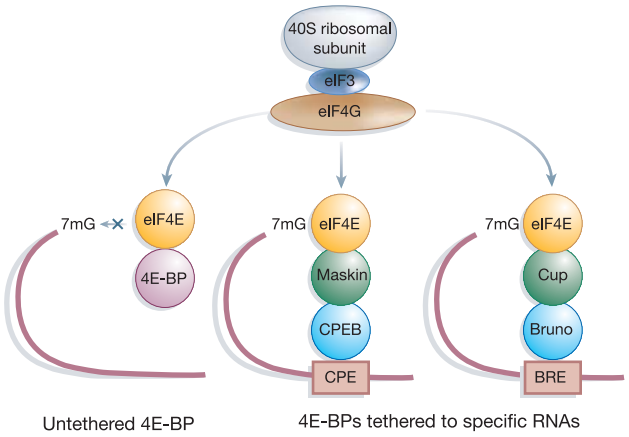

Figure 1 Translational control by eIF4E inhibitory proteins. Translation initiation occurs when the 40S ribosomal subunit is recruited to the 5′ ends of capped (that is, 7mG-containing) mRNAs through an eIF3–eIF4G–eIF4E interaction. Initiation is disrupted by 4E-BP, which binds and sequesters eIF4E by interacting with eIF4G; this process occurs on a number of mRNAs because 4E-BP is not tethered to any particular sequence. Two examples of tethered 4E-BPs are represented by Maskin (*Xenopus*) and Cup (*Drosophila*). Through its association with CPEB, Maskin interacts with the eIF4E only on RNAs that contain a cytoplasmic polyadenylation element (CPE); disruption of the eIF4E–eIF4G complex by this protein is therefore mRNA-specific. In a similar manner, Cup, through its association with Bruno, binds and displaces the eIF4G from eIF4E only on mRNAs that contain a Bruno response element (BRE). Note, however, that Cup also binds Smaug, a protein that binds *nanos* mRNA; thus Cup associates with the eIF4E on this mRNA as well.

soluble protein stimulated by insulin) form nearly identical α-helical structures that lie along the same convex region of eIF4E, some distance from this protein's cap binding site[3,4]. Peptides with the general sequence YXXXXLϕ, where ϕ is any hydrophobic amino acid, would probably form similar α-helical structures, implying that other proteins containing this peptide motif could control translation initiation.

The original three eIF4E inhibitory proteins, the 4E-BPs, prevent eIF4F complex formation by sequestering available eIF4E (ref. 5). This sequestration results in the inhibition of translation of certain mRNAs that normally require high levels of available eIF4E (ref. 6). The newly discovered eIF4E-binding proteins described below interact with the eIF4E on only specific mRNAs, and do so either because they also interact with certain RNA elements directly, or do so through affiliations with RNA binding proteins.

eIF4E inhibitory proteins in development

One characteristic of early animal development is the synthesis and storage of mRNAs that are destined for later use. Although no single mechanism regulates the translation of all these mRNAs, one of the most important is the modulation of poly(A) tail length. For example, the 3′ ends of most mRNAs terminate with ~150–200 adenylate residues that generally enhance stability and translation. In frog oocytes arrested at the end of meiotic prophase I, however, several dormant but stable mRNAs have relatively short poly(A) tails of approximately 20–40 bases. When the oocytes are stimulated to re-enter the meiotic divisions in preparation for fertilization, the poly(A) tails on these mRNAs are elongated to about 150 bases and translation ensues. Cytoplasmic polyadenylation is controlled by CPEB, a protein that interacts with the cytoplasmic polyadenylation element (CPE), a small sequence in the 3′ UTR of mRNAs. CPEB also binds Maskin, a protein that interacts rather weakly with eIF4E probably because it contains an unusual threonine (T) for tyrosine (Y) substitution in its eIF4E-binding domain. In spite of this weak binding, Maskin disrupts eIF4E–eIF4G interactions; the CPEB–Maskin–eIF4E complex therefore inhibits the translation of CPE-containing mRNAs specifically[7]. When frog oocytes are induced to complete meiosis, CPEB stimulates poly(A) tail growth; the newly elongated poly(A) tail then is bound by poly(A) binding protein (PABP), which in turn interacts with eIF4G. PABP-bound eIF4G then displaces Maskin from eIF4E, thereby inducing translation[8] (Fig. 2).

Complexes that are functionally equivalent to the CPEB–Maskin–eIF4E trimer have recently been uncovered in *Drosophila*, where the asymmetric distribution of mRNAs and proteins in the egg determine the body plan in the embryo. In the anterior portion of the embryo, Bicoid, a homeobox-containing transcription factor, activates genes that control segmentation; in that region, Bicoid also represses translation of the mRNA encoding Caudal, a transcription factor that directs the formation of posterior structures. Bicoid not only binds a small sequence in the *caudal* mRNA 3′ UTR (the Bicoid binding region or BBR), it is also retained on an affinity matrix composed of cap-bound eIF4E (ref. 9). Although the Bicoid used for these experiments was derived from a cell extract that could contain several cap binding proteins, it may have directly associated with eIF4E, possibly through a peptide motif that resembles those in the 4E-BPs and Maskin[9]. Through these associations, Bicoid may act as a composite of both CPEB and Maskin in that its binding to the BBR confers mRNA-specificity to possibly prevent eIF4E–eIF4G interactions, and thus repress translation.

Three recent studies have identified the protein Cup as a new Maskin-like molecule that controls germ-cell formation and axis specification in *Drosophila*. One substrate that is acted upon by Cup is *oskar* mRNA, which is synthesized in nurse cells and transported through cytoplasmic bridges (ring canals) to the oocyte, where it localizes to the posterior region and is translated. The protein Bruno is responsible for suppressing *oskar* mRNA translation during transport, and does so not only through its association with the Bruno response element (BRE) in the 3′ UTR, but also through an interaction with Cup, an eIF4E-binding protein[10]. The Cup–eIF4E interaction, like the CPEB–Maskin interaction, prevents assembly of the eIF4F complex and thereby inhibits translation. Cup may also function in correct mRNA localization through an additional interaction with Barentsz, a protein that binds the molecular motor kinesin[11].

A second RNA, whose expression is controlled by Cup, encodes Nanos, an RNA binding protein that represses *hunchback* mRNA translation in the posterior pole; this repression is necessary for the proper development of the abdomen and other posterior structures. Whereas some *nanos* mRNA is highly concentrated at the posterior pole, most of it is dispersed throughout the rest of the embryo. This dispersed *nanos* mRNA pool is translationally repressed, at least in part, by Smaug, a protein that binds a *cis* element in the *nanos* 3′ UTR. Smaug also interacts with Cup, which as noted above binds eIF4E to the exclusion of eIF4G, repressing translation[12].

eIF4E inhibitory proteins in cancer

The eIF4E–4E-BP interaction has important implications for disease, including cancer. This is not surprising given that overexpression of eIF4E not only causes malignant conversion of rodent fibroblasts[13] and human mammary epithelial cells[14], but also promotes tumour formation in transgenic mice[15,16]. Consistent with the oncogenic potential of eIF4E, several studies have shown that human tumours express abnormally high levels of this protein[17]. Because the 4E-BPs inhibit eIF4E activity, it would not be surprising if they acted as tumour suppressors. Indeed, ectopic expression of the 4E-BPs in transformed cells partially reverts their transformed phenotype[18]. Interestingly, in several transformed mammary cell lines the 4E-BPs are hyperphosphorylated, which results in their dissociation from eIF4E and, in effect, increases the active concentration of this initiation factor[14]. It is of particular interest that

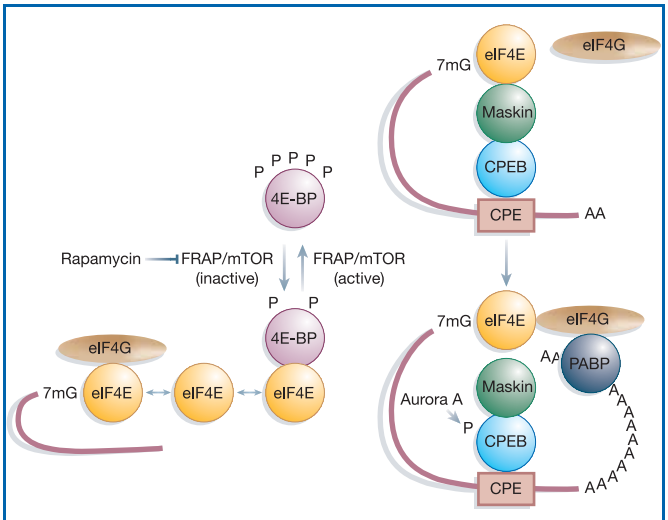

Figure 2 Regulation of eIF4E inhibitory proteins. The kinase FRAP/mTOR hyperphosphorylates 4E-BP on several sites; this causes the liberation of eIF4E from 4E-BP, and the association of eIF4E with both capped mRNA and eIF4G. The inhibition of FRAP/mTOR by rapamycin leads to the hypophosphorylation of 4E-BP and enhanced binding to eIF4E. Maskin binding to eIF4E excludes the eIF4G–eIF4E interaction on CPE-containing mRNAs. The inhibition of translation by Maskin is abrogated by cytoplasmic polyadenylation, which is induced by Aurora A-catalysed CPEB phosphorylation. The newly elongated poly(A) tail is bound by poly(A) binding protein, whose association with eIF4G helps disrupt the Maskin–eIF4E complex and facilitate initiation. The compartmentalization of Maskin- (or Cup-) bound eIF4E probably makes it resistant to further regulation by 4E-BP.

rapamycin, which specifically inhibits the kinase FRAP/mTOR and thus prevents 4E-BP hyperphosphorylation, is currently being tested as an anti-cancer agent[19] (Fig. 2). Along the same lines, compounds that mimic the anti-translational activity of the 4E-BPs might also serve as useful agents to treat malignancies where eIF4E is abnormally high.

An increase in eIF4E amount or activity does not lead to elevated rates of global translation, but instead results in increased translation of a subset of mRNAs. These observations can be explained if cells contain an amount of eIF4E that is just enough to form sufficient eIF4F to maintain basal levels of translation[20] (but see also ref. 21). Because some mRNAs require more eIF4F owing to strong secondary structure in the 5′ UTR, over-expression of eIF4E, which binds excess eIF4G and eIF4A (a factor that melts secondary structure), can selectively result in their increased translation[22]. This is clearly the case with ectopically expressed reporter mRNAs containing excessive secondary structure[23], and also with endogenous mRNAs encoding such proteins as Myc, fibroblast growth factor (FGF), ornithine decarboxylase (ODC) and vascular endothelial growth factor (VEGF)[6,24]. These and other mRNAs play important roles in controlling cell growth and proliferation, and thus the marked effects of eIF4E on transformation could be explained by their elevated rates of translation.

eIF4E inhibitory proteins in the nervous system

Perhaps the newest frontier where translational control by the 4E-BPs is likely to have an enormous influence is the central nervous system. One neuronal activity that relies on protein synthesis is synaptic plasticity, which may be the underlying molecular and cellular basis for long-term memory consolidation. Synapses, the structures neurons use to communicate with one another, are plastic because they undergo biochemical and morphological modifications following their activation. These modifications are used by neurons to mould the strength of their responses. For these responses to endure, a neuron must establish a 'tag' at the stimulated synapse and then recognize and respond to it when the synapse undergoes subsequent stimulations[25]. Although the nature of the tag(s) is not yet defined, its establishment or recognition requires protein synthesis, at or in close proximity to the synapse. Many different mRNAs are present in dendrites, but it is not clear how many are recruited for translation following synapse stimulation, nor is it certain which may be involved in synaptic tagging[26,27]. Studies using rapamycin indicate that FRAP/mTOR signalling is involved in establishing the tag[28,29]. Moreover, 4E-BPs are detected at synapses, suggesting that they may be the substrates of FRAP/mTOR signalling[30]. Current studies using knockout mice should help to distinguish between the effects of rapamycin on 4E-BPs or other FRAP/mTOR substrates such as S6 kinase. In other studies, CPEB knockout mice have been found to have a deficit in some forms of synaptic plasticity[31]. These results, plus the observation that CPEB and Maskin immunoreactivity is detected at synapses[32,33], suggest that these molecules influence plasticity by controlling local CPE-dependent translation. However, whether mammals contain functional Maskin (or Cup) has not been demonstrated.

Finally, axons may rely on regulated mRNA translation to find their way to the appropriate destination. In *Xenopus*, protein synthesis inhibitors and rapamycin abrogate the attractive and repulsive turning of isolated retinal growth cones to netrin-1 and semaphorin 3A (ref. 34). These signalling agents also induce 4E-BP phosphorylation, suggesting that growth cone turning is mediated by cap-dependent translational control[34].

Outlook

By deciphering the mechanisms of translational control by eIF4E inhibitory proteins, new insights into how molecular decisions are made in normal and abnormal cells have been revealed. We have focused on development, cancer and neurobiology, three areas in which translational control by these proteins clearly has a great impact, and where we think significant discoveries will yet be made. There has also been substantial progress in understanding how eIF4E inhibitory proteins influence cell-cycle progression and metabolism[35–37]. Whereas recent studies report that several mammalian transcription factors can bind eIF4E (refs 38, 39), future experiments may uncover new eIF4E inhibitory proteins and demonstrate their importance in diverse biological processes. □

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