Control of protein translation by phosphorylation of the mRNA 5′-cap-binding complex

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Abstract

Initiation of mRNA translation is a key regulatory step in the control of gene expression. Microarray analysis indicates that total mRNA levels do not always reflect protein levels, since mRNA association with polyribosomes is necessary for protein synthesis. Phosphorylation of translation initiation factors offers a cost-effective and rapid way to adapt to physiological and environmental changes, and there is increasing evidence that many of these factors are subject to multiple regulatory phosphorylation events. The present article focuses on the nature of reversible phosphorylation and the function of the 5′-cap-binding complex in plants.

Introduction

Gene expression can be regulated at numerous levels from alterations in the chromatin state of the gene through to transcription of DNA, processing and translation of the mRNA to post-translational modification of the protein. At the post-transcriptional level, translation of mRNA is a key target of regulation by 5′- and 3′-UTR (untranslated region)-binding factors [1]. In specialized cells lacking transcriptional controls, such as oocytes and early embryos where maternal mRNA prevails, translational control seems to be the primary regulatory mechanism [2].

Global transcriptome and proteomic analyses indicate that mRNA abundance does not always correlate with protein levels [3]. Such discrepancies can arise due to variable translation of transcripts and either can be restricted to specific post-transcriptionally regulated genes or can be general in scope. Changes in translation activity often occur in response to stress or lack of nutrients [4], during mitosis [5], cell and organ development [6], embryogenesis [7] and oncogenesis [8]. The ability of mRNA to be translated depends on its association with polyribosomes, and the partitioning of an mRNA between monosomes and polysomes is often used as a measure of its potential 'translatability' [9].

The phosphorylation of proteins involved in translation, such as ribosomal proteins [10], eIFs (eukaryotic initiation factors) [11] or elongation factors [12], as well as the PABP [poly(A)-binding protein] [13] contribute to the overall regulation of gene expression at the level of protein synthesis. In the present article, we focus on the phosphorylation of factors involved in the initiation of protein synthesis, with an emphasis on components of the cap complex in plants. Importantly, these phosphorylation events allow the coupling of protein translation and other essential cellular functions such as the stress response, cell growth and division.

Protein translation initiation complexes

Eukaryotic translation initiation is a complex series of events that are assisted by more than 25 polypeptides [14]. Translation is initiated when a particular mRNA is loaded on to the 43S pre-initiation complex formed by association of the 40S ribosomal subunit with several eIFs (eIF1, eIF3 and eIF5) and the tRNA^{met}–eIF2 complex [14]. The eukaryotic initiation surveillance complex is composed of at least five eIFs (eIF4E, eIF4G, eIF4A, eIF4B and eIF3) and PABP. Additional factors seem to be specific to animals or plants: in animals, eIF4H (like eIF4B) regulates the helicase activity of eIF4A [15], whereas plants possess two novel eIF4 isoforms, eIFiso4E and eIFiso4G [16]. By promoting the loading of the mRNA on to the pre-initiation complex, the initiation surveillance complex ensures that only intact mRNAs are recruited for translation and serves to enhance the fidelity of protein synthesis [11]. In a simple model (Figure 1), the cap-binding protein eIF4E binds to the large eIF4G scaffold protein and the 5′-m^{7}GpppG [7-methylguanosine(5′)triphospho(5′)guanosine] cap of the mRNA, which allows the binding of the RNA helicase eIF4A and its regulators eIF4B or eIF4H [15]. The assembly of the eIF4 complex enhances mRNA cap-binding and RNA helicase activities of eIF4E and eIF4A respectively. The eIF4 cap complex interacts with the pre-initiation complex via interactions between both eIF4G and eIF4B with eIF3. Another key component is PABP, which, through interactions with both the poly(A) tail of the transcript, eIF4G, and eIF4B, promotes the circularization of the mRNA [17]. The helicase function of eIF4A serves to unwind secondary structures in the 5′-UTR to facilitate scanning of the 43S ribosomal subunit until the initiation codons (AUG), whereupon the 60S ribosomal subunit binds and translation elongation then occurs.

Key words: 5′-cap-binding complex, initiation factor, mRNA, post-translational modification, protein–protein interaction, translation regulation.

Abbreviations used: CDK, cyclin-dependent kinase; eIF, eukaryotic initiation factor; eIF4E-BP, eIF4E-binding protein; MAPK, mitogen-activated protein kinase; Mnk, MAPK-interacting kinase; PABP, poly(A)-binding protein; PKC, protein kinase C; PKR, double-stranded-RNA-dependent protein kinase; TOR, target of rapamycin; UTR, untranslated region.

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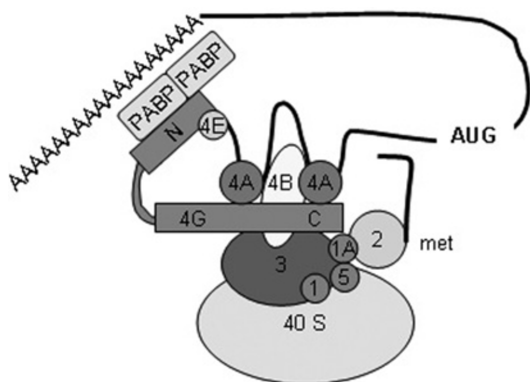
Figure 1 | Schematic representation of the organization of translation initiation factors on the capped mRNA

The translation initiation factors depicted are either part of the initiation surveillance complex (eIF4 cap complex, eIF3 and PABP) or/and part of the 43S pre-initiation complex (40S, eIF1, eIF1A, eIF2, eIF3 and eIF5).



If scanning and elongation proceed, then additional ribosomes can be sequentially loaded on the same transcript. A genome-wide analysis of mRNA translation profiles in yeast [18] has shown that most of the mRNAs were associated with a variable number of ribosomes (between 1 and 15) and that transcripts of translationally regulated genes tended to peak in the monosome fraction. The number of ribosomes bound to each transcript tends to increase as the length of the translated sequence increases, but ribosomes were still well spaced on nearly all mRNAs, suggesting that initiation is the rate-limiting step in translation.

Variation in localization and composition of the eIF4 cap complex

The cellular localization of translation initiation factors is predominantly cytoplasmic [19]. However, in addition to its cytoplasmic localization, eIF4G was also found in the nucleus of mammalian cells *in vivo*, associated not with eIF4E, but with the nuclear cap-binding complex [20]. Therefore assembly of the initiation surveillance complex on the 5′-cap of the mRNA may start in the nucleus during transcription and RNA processing and facilitate processing and transport of the mRNA to the cytoplasm for translation [20]. Little is known of the localization of plant initiation surveillance complex components; it is assumed that, as in animals, cytoplasmic and nuclear pools of eIF4E and eIF4G coexist.

Although the components of the cap complex seem to be fairly conserved between animals and plants, there are kingdom-specific isoforms [21]. Moreover, it is likely that the cap complex composition also varies according to the cell types, tissues or environmental conditions. Variations in composition have been observed in responses to physiological changes or modulation of signal transduction pathways. Research in our laboratory has revealed that composition of the cap complex varies according to the growth stage of *Arabidopsis* cell suspension, shifting from a complex array of proteins in proliferating cells to a simple selection in quiescent cells (M.S. Bush and J.H. Doonan, unpublished work). Depending on the subunit, the variation could be due to protein turnover, but, in some cases, recruitment into the complex also plays an important role. Regulation of cap complex components could therefore provide an additional layer of regulation.

Reversible phosphorylation of translation initiation factors

Recruitment of components into the cap complex could depend on protein phosphorylation. Reversible protein phosphorylation is a very widespread regulatory mechanism for controlling protein activity in eukaryotes. It is also remarkably cost-effective in that activity of proteins can be turned on and off without *de novo* synthesis and degradation. Also, multiple phosphorylation events leading to contradictory and complex effects provides a mechanism whereby translation can be fine-tuned according to multiple input signals.

The α subunit of mammalian eIF2 is phosphorylated on Ser^{51} by several kinases such as the HRI (haem-regulated inhibitor of translation), the PKR (double-stranded-RNA-dependent protein kinase, also known as dsRNA-PK) and the PERK (PKR-like endoplasmic reticulum protein kinase) [22,23]. In mammalian cells, this phosphorylation enhances the binding of eIF2 to eIF2B, preventing GTP/GDP exchange and inhibiting translation [24]. A similar mechanism may be present in plants, but has not yet been proved [25]. Mammalian eIF4E is phosphorylated on Ser^{209} by Mnk [MAPK (mitogen-activated protein kinase)-interacting kinases] 1 and 2, cPK (insulin-stimulated protamine kinase) and PKC (protein kinase C), in response to various stimuli such as growth factor or stress [26–28]. Mammalian eIF4G provides a docking site for Mnk1 and Mnk2 that brings them to their eIF4E substrate [28]. *In vitro* phosphorylation of the plant-specific eIFiso4E at Ser^{207} by protein kinase CK2 reduced its cap-binding affinity [29]. Mammalian eIF4G is phosphorylated by many kinases: through the phosphoinositide 3-kinase and FRAP (FK506-binding protein 12/rapamycin-associated protein)/TOR (target of rapamycin) pathway [30] in response to serum stimulation; by the S6 kinase, PKC and PAK2 (p21-activated protein kinase 2) [31,32]; and by the CaMKI (Ca^{2+}/calmodulin-dependent protein kinase I) at Ser^{1156} [33]. eIF4B is also a target of the S6 kinase [34] and the Pim kinase [35], its phosphorylation status being regulated by the S6/mTOR (mammalian TOR) and MAPK pathways [36]. Initial attempts to identify phosphorylation of eIF4A showed that eIF4A was predominantly non-phosphorylated [6]. However, a phosphorylated form of eIF4A was found in wheat and maize under stress conditions such as hypoxia [37,38]. Although tobacco pollen contains multiple isoforms of eIF4A, only two are phosphorylated during pollen germination, of which one is the pollen-specific isoform, eIF4A8 [39]. Clearly, these observations suggest a subtle regulation of eIF4A by phosphorylation in response

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to different environmental and physiological conditions [11]. To our knowledge, there is no evidence that eIF4A is phosphorylated in mammals or yeast. Phosphorylation of the plant PABP dictates its co-operative binding to poly-adenylated RNA and its interaction with other eukaryotic initiation factors [13]. In humans, a novel signalling pathway involving MKK-2 (MAPK kinase 2) and ERK (extracellular-signal-regulated kinase) 1/2 may down-regulate the activity of PABP and eIF4E by controlling their phosphorylation and compensate for the effect of excess cellular PABP [40].

Regulatory role of the translation initiation factor phosphorylation

The multiplicity of phosphorylation events on initiation complexes suggests that they are more likely to be regulatory than essential, as they are not essential for protein synthesis *in vitro* and *in vivo* [41]. Phosphorylation of many initiation factors is induced by abiotic and biotic stress signals. In animals and plants, eIF2, eIF4E, eIF4A and eIF4B were found to be hyperphosphorylated after stress conditions such as haem deprivation, nutrient depletion, hypoxia, heat shock or viral infection [38,42–43]. The phosphorylation state of initiation factors varies also during cell growth and organ development. Since growth factor signalling tends to trigger a branched cascade of phosphorylation, the identity of the kinases that directly phosphorylate eIFs is of great interest. For instance, the hormone-induced meiotic maturation of the *Xenopus* oocyte is regulated by complex changes in the phosphorylation of eIF4 proteins and results in increased translation activity [44]. Phosphorylation of eIF4E is increased in response to treating cells with serum, growth factors, phorbol esters and, in some cell types, insulin [45]. During seed germination in wheat, the α and β eIF2 subunits dynamically change their phosphorylation status [6]. Finally, there is growing evidence that phosphorylation status can vary in relation with the cell cycle. During mitosis in mammals, phosphorylation of the eIF4E-BPs (eIF4E-binding proteins) by the CDK (cyclin-dependent kinase) 1 results in their dissociation from eIF4E, allowing eIF4E to form translation-active eIF4F complexes [46]. There is no obvious homologue of eIF4E-BP in plants, but alternative interactions exist between CDKs and eIF4 components [47]. During mitosis in animal cells, eIF4E is dephosphorylated and eIF4GII is hyperphosphorylated leading to eIF4F complex disruption, whereas cap-independent translation of specific mitotic-regulated genes is favoured to promote key mitotic functions such as cytokinesis [5]. Recently, the tumour suppressor 14-3-3 σ factor was identified as a regulator of mitotic translation through its direct mitosis-specific interaction with eIF4B. Cells lacking 14-3-3 σ cannot suppress cap-dependent translation and do not stimulate cap-independent translation during and immediately after mitosis [48].

The functional consequences of such phosphorylations are not straightforward to unravel, are still not fully understood and are subject to controversy. Indeed, phosphorylation of different sites can result in either enhanced or decreased protein translation, according to the type of initiation factor and conditions. Biochemical data from mammals and plants showed that phosphorylations of eIF4E and eIFiso4E decrease affinity for the capped RNA, suggesting rather an inhibition of the cap-dependent translation [29,49]. Phosphorylation of eIF4A in response to heat shock may provide an adaptive response to prolonged stress [38] and may not be an immediate reaction to the stress. However, eIF4B dephosphorylation observed following heat shock, serum depletion or mitosis correlates with a reduction in translation, whereas insulin-stimulated phosphorylation has the opposite effect [38].

Conclusions

We have reviewed some key examples indicating that protein phosphorylation of initiation factors plays a central role in controlling the level of protein synthesis. Phosphorylation of translation factors provides an effective way of regulating protein synthesis to adapt to changes during stress or development and may allow the selective translation of certain mRNAs under specific conditions. A recent study [50] reported a coupling between the transcriptional activation of specific mRNAs in response to nutritional stress and a cap-independent translation mechanism. Likewise, the interplay between translational control and the cell cycle remains to be fully elucidated: *de novo* production of cyclins and many other proteins necessary for mitosis must occur at a time when the cell is shutting off general translation [51]. Sucrose starvation in *Arabidopsis* [52] indicates there is striking differences in mRNA abundance and in polysomal representation suggesting that translational control is important for many cyclical and dynamic processes. Understanding the effect of phosphorylation events on one hand and structural features in the different transcripts on the other should provide some insight into the underlying molecular mechanisms.

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