TRANSLATIONAL CONTROL BY CPEB: A MEANS TO THE END

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The regulated translation of messenger RNA is essential for cell-cycle progression, establishment of the body plan during early development, and modulation of key activities in the central nervous system. Cytoplasmic polyadenylation, which is one mechanism of controlling translation, is driven by CPEB — a highly conserved, sequence-specific RNA-binding protein that binds to the cytoplasmic polyadenylation element, and modulates translational repression and mRNA localization. What are the features and functions of this multifaceted protein?

POLYSOMES
Complex of ribosomes bound to a single messenger RNA molecule.

GERM LAYERS
Embryonic cell layers (endoderm, mesoderm and ectoderm) from which the embryonic organs and structures are derived.

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It seems time that one precept of molecular biology — that transcription is the master switch of gene expression whereas translation merely fine-tunes when and where proteins are made — be put to rest. The past few years have witnessed a veritable explosion in the number of examples in which translational control is crucial for determining cell function. Take, for example, early development, during which the temporal and spatial activation of messenger RNAs is necessary for cell division and proper body patterning. In invertebrates such as *Drosophila melanogaster*, several mRNAs that are synthesized during oogenesis and inherited by the fertilized egg (that is, maternal mRNAs) are translated in the embryo in an enormously complex pattern. The products of some of these translationally controlled mRNAs are themselves translational regulators; this hierarchy of regulation directs axis specification of the developing animal and the formation of pole cells, which contain the material that programmes the germ cells of the succeeding generation.

Vertebrate development is also directed by maternally inherited mRNAs that are synthesized and stored during the long period of oogenesis. In *Xenopus laevis*, for which most of the molecular details have been worked out, many maternal mRNAs are dormant in oocytes, and their mobilization into POLYSOMES does not occur until later in development. One of these times is oocyte maturation, the late stage of meiosis that immediately precedes fertilization, when mRNAs encoding several cell-cycle-control proteins are translated. Another time is during early embryogenesis, when the establishment of GERM LAYERS requires the translation of several other mRNAs.

But the developing embryo is not the only place where important decisions are made at the translational level — consider the somatic translational control of ferritin[1] and lipoxygenase[2] mRNAs, or the critical role of translational regulation in the central nervous system. Here, one neuron might have a thousand or more inputs from axons that emanate from other neurons; but when a group of these axons, or their synaptic connections, is stimulated, the receiving neuron responds and 'remembers' which synapses were the ones that were stimulated. This 'memory' takes the form of synaptic plasticity, because when the stimulated synapse is stimulated again, its strength of response (or synaptic efficacy) is different from its first response. How does the neuron remember? This is certainly a complex process, but it seems clear that it involves the translational activation of mRNAs present near the synapses, in dendritic spines or shafts.

A primer of translation initiation

Because translational control mechanisms ultimately affect the basic protein synthesis machinery, a familiarity with some of the key components is essential for understanding regulation (see the review by LaFontaine and Tollervey on page 514). The control of translation is most often exerted at the initiation

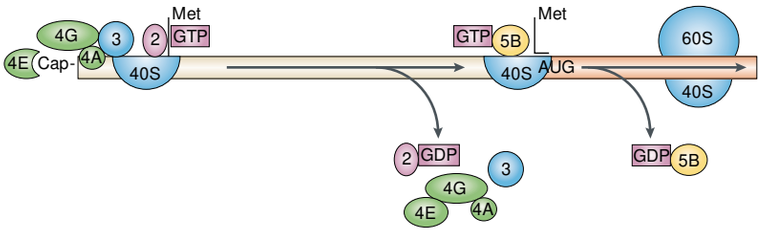


Figure 1 | **Translational initiation in eukaryotes.** The 43S initiation complex is composed of the cap-binding complex (eIF4E, eIF4G and eIF4A), eIF3, the ternary complex (eIF2, Met–tRNA and GTP) and the 40S ribosomal subunit. Once recruited to the cap structure, the 43S complex scans towards the 3′ end until it reaches the initiation codon (AUG). At this point, the initiation factors are released and the 60S ribosomal subunit is recruited to the mRNA with the assistance of eIF5B. This model is simplified for clarity and not all the initiation factors are depicted. Also, the precise time at which particular initiation factors (such as eIF4E and eIF4G) are released from the 43S complex is not yet well characterized.

eIF3
Eukaryotic translation initiation factor that mediates dissociation of the 40S and 60S ribosomal subunits and the recruitment of the Met–tRNA–eIF2 complex and the 40S ribosomal subunit to the messenger RNA.

UTR
Non-coding portions of the messenger RNA that precede the starting codon (5′ UTR) or follow the termination codon (3′ UTR).

step, during which binding of the 40S ribosomal subunit to the mRNA is rate limiting.

FIGURE 1 shows the elemental features of initiation for most cellular mRNAs[3]. The cap structure (m^7GpppN) at the 5′ end of the mRNA facilitates ribosome binding through an interaction with the cap-binding protein complex. This complex comprises three subunits: the cap-binding protein eIF4E; the RNA helicase eIF4A; and the modular scaffold protein eIF4G. eIF4G not only binds both eIF4E and eIF4A, but it also forms a bridge between the ribosome and the mRNA through an interaction[4,5,6] with eIF3. eIF4G also binds the poly(A)-binding protein (PABP), which facilitates the translation of poly(A)-containing mRNAs[7,8,9].

The cap-binding complex, in combination with yet another factor, eIF4B, unwinds secondary structure in the 5′ untranslated region (UTR) of the mRNA. This helps the 40S ribosomal subunit pass through this region unimpeded. During this transit, eIF3 is bound to the 40S PRE-INITIATION COMPLEX with eIF2, which forms a ternary complex with GTP and the transfer RNA that is charged with the initiation methionine (Met–$tRNA_i$). This large 43S complex then scans towards the initiation AUG codon, which is recognized by the anticodon of the Met–$tRNA_i$. At this point, the GTP bound to eIF2 is hydrolysed, the initiation factors are released, and the 60S subunit joins the 40S (for which it requires eIF5B–GTP[10]). In this now 80S MONOSOME, eIF5B is released as eIF5B–GDP, and the elongation phase of translation begins.

Negative regulators of translation often interfere with the assembly of the 48S initiation complex (the mRNA-associated 43S complex). Such factors include the eIF4E-binding proteins (eIF4EBPs)[11], which do not discriminate among mRNAs, or others that are tethered — directly or indirectly — to specific sequences in the 5′ or 3′ UTRs. Such mRNA-specific regulators include the iron regulatory protein (IRP)[1,12] and maskin[13] (see below). Conversely, a positive regulator of translation, PABP, potentiates the assembly of the 48S complex[7,14].

Compounding this complexity of regulation is the cellular milieu in which it occurs, because mRNAs are

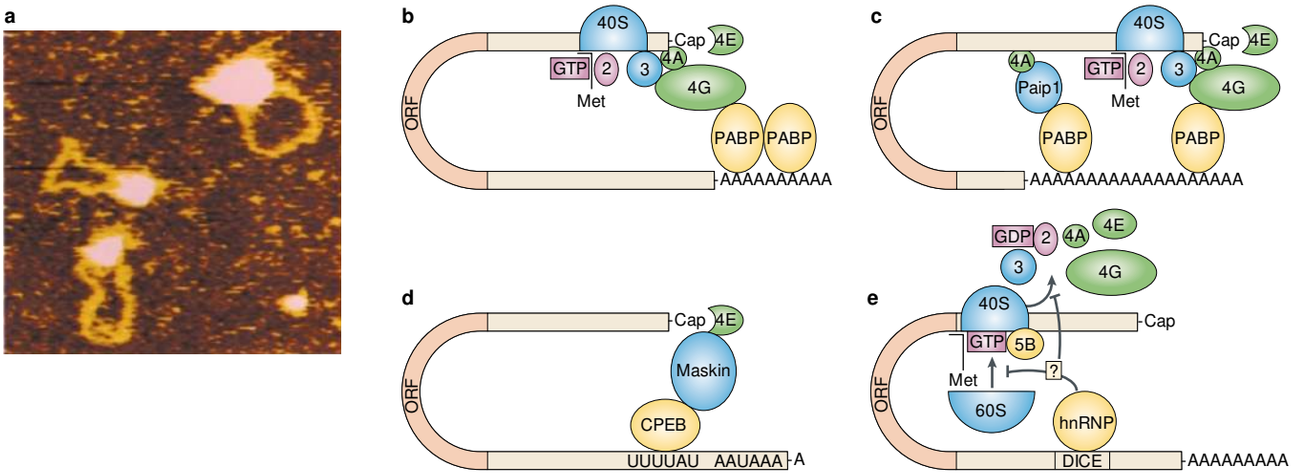
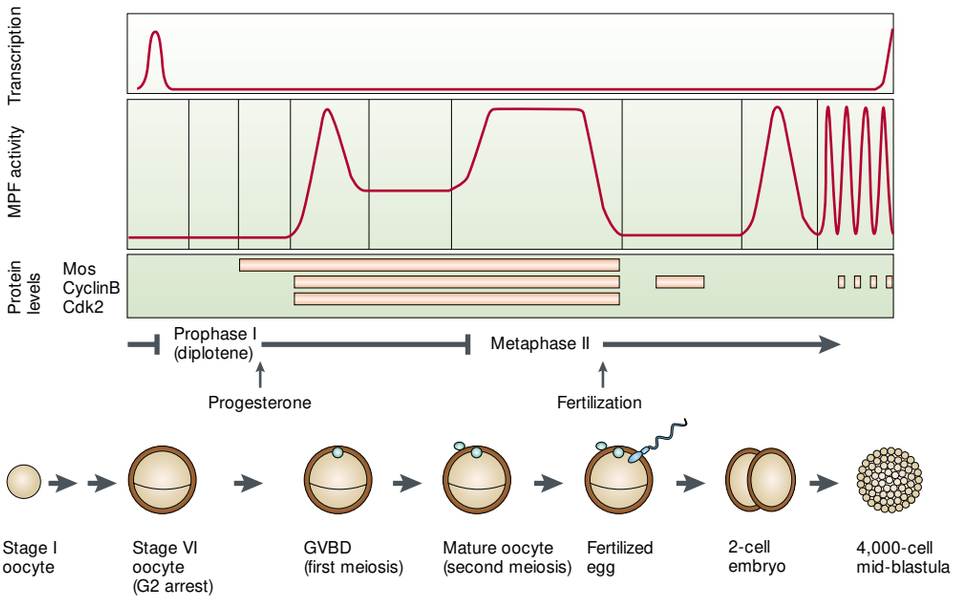


Figure 2 | **3′–5′ interactions: circles of mRNA. a** | Visualization of circular RNA–protein complexes by atomic-force microscopy. Complexes formed on capped, polyadenylated double-stranded RNA in the presence of eIF4G, poly(A)-binding protein (PABP) and eIF4E[91]. (Picture provided by A. Sachs and reprinted with permission.) **b** | Model of messenger-RNA circularization and translational activation by PABP–eIF4G–eIF4E interactions. eIF4G simultaneously binds to eIF4E and PABP[7,9,14,53,55], thereby circularizing the mRNA[91] and mediating the synergistic stimulatory effect on translation of the cap and poly(A) tail by enhancing the formation of the 48S complex[53,54,92]. **c** | Model of mRNA circularization and translational activation by PABP–Paip1 interactions. Paip1 is a PABP-interacting protein that binds eIF4A[93], acting as a translational co-activator. **d** | Model of mRNA circularization and translational repression by CPEB–maskin–eIF4E interactions. RNA-associated CPEB binds maskin, which in turn binds to the eIF4E. This configuration of factors precludes the binding of eIF4G to eIF4E and thus inhibits assembly of the 48S complex[13]. **e** | Model of translational repression by heterogeneous nuclear ribonucleoproteins (hnRNPs). The differentiation control element (DICE), located in the 3′ UTR of 15-lipoxygenase mRNA, inhibits translation initiation by preventing the joining of the 60S ribosomal subunit to the 43S complex located at the AUG codon. This inhibition is mediated by hnRNP proteins K and E1. The inhibitory event probably targets one of the initiation factors involved in the GTP hydrolysis that releases the initiation factors and the joining of the 60S ribosomal subunit[2,94]. ORF, open reading frame.



Transcripts

Actin	—AAAAAA	—A	—A	—A		
Cyclins A1, B1 and B2	—A	—AAAAAA	—AAAAAA	—AAAAAA	—AAAAAA	
Cl2, Cl1	—A	—A	—A	—A	—AAA	—AAAAAA
Eg2, Cdk2 c-mos	—A	—AAAAAA	—AAAAAA	—A		

Figure 3 | **Key events during *Xenopus laevis* oocyte maturation and early embryogenesis.** The upper panel shows the relative rate of messenger RNA transcription, M-phase promoting factor (MPF) activity, and the levels of the Mos, cyclin B and cyclin-dependent kinase 2 (Cdk2) proteins during early development. The lower panel depicts the state of poly(A) growth or removal of several mRNAs during this same time period.

40S PRE-INITIATION COMPLEX
Ribonucleoprotein particle that includes the transfer RNA, the 40S ribosomal subunit and the eIF2.

eIF2
Eukaryotic translation-initiation factor that mediates the recruitment of the Met–tRNA$_i$ to the 40S ribosomal subunit.

eIF5B
Eukaryotic translation-initiation factor that mediates recruitment of the 60S ribosomal subunit to the mRNA-associated 40S ribosomal subunit.

MONOSOME
Single ribosome bound to a mRNA.

hnRNPs
Proteins that bind the heterogeneous nuclear RNA (hnRNA) and that are involved in splicing, RNA transport and translation.

PROPHASE
Initial phase of the cell cycle (mitosis or meiosis), in which the chromatin is condensed. Meiosis contains two prophases not separated by a DNA-replication event.

METAPHASE
Phase of the cell cycle (mitosis or meiosis) in which the nuclear membrane breaks down and the chromosomes are arranged on the equator of the spindle. Meiosis contains two metaphases not separated by a DNA-replication event.

CDC2
Serine/threonine kinase that constitutes the catalytic subunit of the M-phase-promoting factor (MPF).

CYCLIN B1
Regulatory subunit of the M-phase-promoting factor (MPF).

PARTHENOGENESIS
Cell division of an egg without fertilization.

usually in excess and compete with one another for the limited protein-synthesis machinery. Consequently, particular features of an mRNA, such as a long 5′ UTR with extended secondary structure, often affect translational efficiency. Although it seems obvious that the 5′ UTR would have an effect on translational efficiency, the most stringent control of translation is provided by the 3′ UTR. From a mechanistic point of view, there are a few well-characterized examples of 3′-UTR-mediated translational control (FIG. 2), one of which occurs in *Xenopus* oocytes and embryos.

Oocyte maturation and early embryogenesis

Fully grown oocytes, which synthesize and store a complex population of mRNAs, are arrested at PROPHASE I (diplotene). Before they can be fertilized, the oocytes must re-enter the meiotic divisions (oocyte maturation), and this is stimulated by progesterone[15,16]. Maturation — which is accompanied by a cessation of transcription and a complex network of translational activation and repression of stored maternal mRNAs — ends at METAPHASE II, in which the oocytes await fertilization before they can complete the final meiotic division and initiate the embryonic cell divisions (FIG. 3). Mitosis in the embryo is unlike any other, for it lacks any appreciable G1 or G2 phase. When the developing embryo is composed of 4,000 cells, the mid-blastula transition occurs and is characterized by several transformations including the lengthening of the cell cycle and the inclusion of G1 and G2, asynchronous cell division and the induction of transcription[17,18].

A key molecule that acts very early in the maturation process is Mos (FIG. 3), a serine/threonine kinase that has several functions[19]. One is to induce the mitogen-activated protein kinase (MAPK) cascade which, directly or indirectly, leads to the activation of M-phase promoting factor (MPF)[20], a heterodimer of cyclin B and CDC2, that is responsible for the many manifestations of maturation such as breakdown of the nuclear envelope (germinal vesicle). Mos also seems to be involved in the translational activation of CYCLIN B1 mRNA[21,22] (however, also see REF. 23). Newly synthesized cyclin B1 is assembled into a small amount of active MPF that induces an auto-amplification loop by activating stored pre-MPF, thus inhibiting DNA synthesis between the two meiotic divisions. Finally, Mos is a component of cytostatic factor (CSF)[24], which arrests maturation at metaphase II to ensure that oocytes do not divide PARTHENOGENETICALLY[25], most probably[26,27] through the activation of the kinase p90^{rsk}.

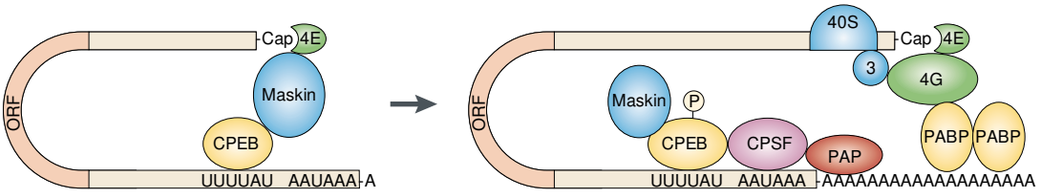


Figure 4 | **CPEB-mediated translational control.** In immature oocytes, messenger RNAs containing a cytoplasmic polyadenylation element (CPE) are translationally dormant (masked) and reside in a complex containing the CPE-binding protein (CPEB), maskin and eIF4E. Once maturation begins, newly phosphorylated CPEB (by the kinase Eg2) recruits the cleavage and polyadenylation specificity factor (CPSF) and poly(A) polymerase (PAP), which elongates the poly(A) tail. At a time coincident with this elongation, maskin dissociates from eIF4E. One possible cause of this maskin–eIF4E dissociation is the formation of a stable poly(A)-binding protein (PABP)–eIF4G complex, which outcompetes maskin for binding to eIF4E and thereby assembles the 48S complex. ORF, open reading frame.

Given these crucial functions of Mos, one might expect oocytes to have an abundance of this protein — in fact, they have none. However, oocytes do contain dormant *mos* mRNA that must be translated for maturation to proceed. The activation of *mos* (and other) mRNA(s) is mediated by cytoplasmic polyadenylation (FIG. 3).

Cytoplasmic polyadenylation

Mos, cyclin B1 and several other dormant mRNAs in oocytes contain short poly(A) tails (~20–40 nucleotides long), and it is only when these tails are elongated (to ~150 nucleotides) that translation takes place. Polyadenylation requires two elements in the 3′ UTR: the hexanucleotide AAUAAA, which is also necessary for nuclear pre-mRNA cleavage and polyadenylation; and the nearby (usually within 20–30 nucleotides) cytoplasmic polyadenylation element (CPE)[17–20].

The sequence of the CPE is variable and includes sequences as diverse as UUUUAU[28–30] to UUUUAACA[31]. However, a general consensus seems to be UUUUUAU. Some mRNAs (for example, cyclin B1) contain many CPEs, and this confers a Mos[21] and cdc2 (REF. 32) dependency on their ability to be polyadenylated. Overall, the precise sequence of the CPE, the number of copies of the CPE, the distance between the CPE and the hexanucleotide, or sequences adjacent to the CPE (such as the nanos response element (NRE) in cyclin B1 mRNA[33]), might regulate the time at which polyadenylation takes place.

The CPE is bound by CPEB (FIG. 4), a highly conserved ZINC FINGER and RNA-RECOGNITION MOTIF (RRM)-type RNA-binding protein[34,35] (BOX 1). The instigation of polyadenylation by this protein requires the kinase Eg2, an enzyme that is activated soon after oocytes are exposed to progesterone[36] and which seems to be further activated at maturation[37]. Eg2, a member of the AURORA family of serine/threonine protein kinases, phosphorylates CPEB at serine residue 174 (REF. 38), an event that increases the affinity of CPEB for the cleavage and polyadenylation specificity factor (CPSF)[39]. However, the Eg2 phosphorylation site does not seem to be present in invertebrates (BOX 1), so if CPEB is to be activated it must be through a different kinase, such as MAPK[40]. CPSF binds to the AAUAAA sequence[41–43], an interaction that is probably stabilized by CPEB, and recruits poly(A) polymerase to the end of the mRNA[39].

Xenopus oocytes, like somatic cells, contain many forms of poly(A) polymerase[44–46]. So which poly(A) polymerase catalyses polyadenylation? One of these forms lacks a carboxy-terminal portion that contains both the NUCLEAR LOCALIZATION SIGNAL and the main cdc2 recognition sites[45]. Such sites become phosphorylated as cells enter M phase and, as a consequence, the polymerase is inactivated[47,48]. The non-truncated poly(A) polymerase, which is both cytoplasmic and nuclear in *Xenopus* oocytes[44], becomes phosphorylated and presumably inactivated as maturation (that is, M phase) proceeds. These observations suggest that the short form of poly(A) polymerase might uniquely catalyse cytoplasmic polyadenylation (FIG. 4).

CPE-mediated translational repression

Because CPE-containing mRNAs are, by and large, inactive in oocytes, it would seem plausible that the CPE is involved in translational repression (masking) as well as polyadenylation. Indeed, a simple injection of CPE-containing RNA into oocytes relieves the translational repression of — unmasks — endogenous CPE-containing cyclin B1 mRNA[49]. In addition, reporter RNAs harbouring a CPE in the 3′ UTR are masked after injection[31,50–52]. This suggests that CPEB is a masking factor as well as a polyadenylation-inducing factor.

Recent evidence indicates that the masking function of CPEB is only an indirect one. Another inhibitory protein called maskin seems to hold the key to how mRNA translation is regulated — it interacts simultaneously with both CPEB and eIF4E[13] (FIG. 4). The interaction between maskin and eIF4E is mediated by an eIF4E-binding motif that is present in all metazoan eIF4Gs as well as other eIF4EBPs. Because of this motif, maskin and eIF4G (and the eIF4EBPs and eIF4G) compete for binding to the same region of eIF4E[5]. Consequently, a competition between maskin and eIF4G for occupancy of eIF4E mediates translation; when maskin is bound to eIF4E, translation (or more precisely, the formation of the eIF4G-requiring 48S complex) is repressed.

ZINC FINGER
Nucleic-acid-binding protein structures containing cysteine or histidine residues at both extremities of the domain, which are involved in the tetrahedral coordination of a zinc atom.

RNA-RECOGNITION MOTIF (RRM). Sequence-specific RNA-recognition domain present in RNA-binding proteins. It consists of ~90 amino acids in α-helical and β-sheet topology, arranged in an αβαββαβ structure.

AURORA KINASES
Family of serine/threonine kinases required for bipolar spindle assembly and chromosome segregation.

NUCLEAR LOCALIZATION SIGNAL
Small stretch of amino acids recognized by the importin protein complex that directs the translocation of the targeted protein through the nuclear pore into the nucleus.

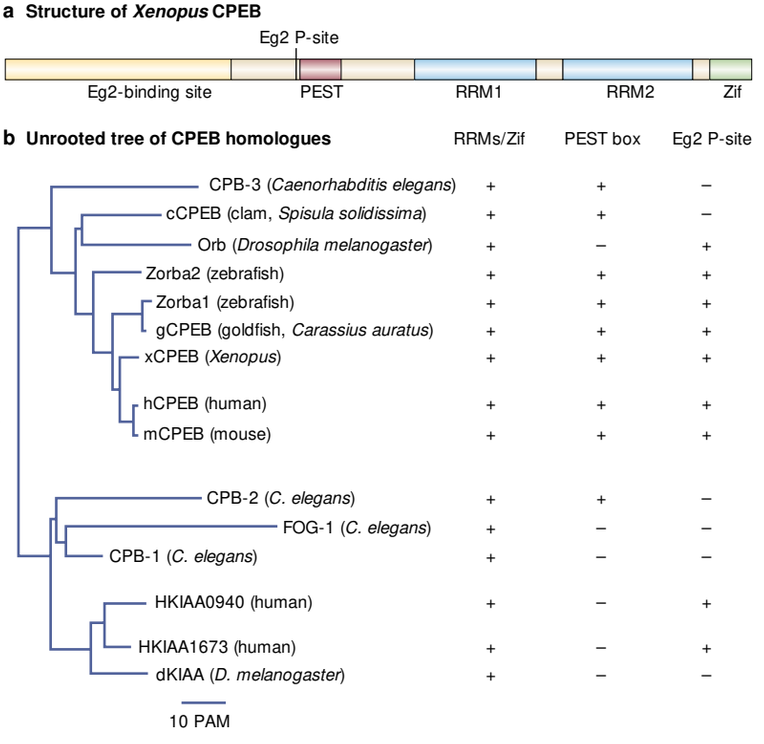
Polyadenylation-induced translation

The foregoing discussion indicates that mRNA unmasking would have to involve the dissociation of maskin from eIF4E. Not only does this dissociation take place (at least partially)[13], but it occurs at a time that is coincident with cytoplasmic polyadenylation. Although these two events might be coincidental, it is provocative to think that polyadenylation could induce translation by causing the dissociation of maskin from eIF4E.

So how could polyadenylation lead to the dissociation of maskin and eIF4E? One attractive possibility is based on the observation that the 5′ cap and the poly(A) tail act synergistically to stimulate translation[53,54]. This synergism might reflect a stabilization of the eIF4E–eIF4G interaction by PABP, which interacts directly with eIF4G (FIG. 4)[7,9,14,55,56]. In the maturing oocyte, the newly elongated poly(A) tails might associate with PABP, which in turn could help eIF4G and eIF4E to form a complex that is more stable than a maskin–eIF4E complex. This would result in the initiation of translation. Although 'classical' PABP seems to be present in low amounts in oocytes[57], there is an oocyte and early embryo form that also contain a putative eIF4G-binding site[58]. Moreover, post-translational modifications of CPEB[38,59,60], eIF4G[61] or perhaps even

Box 1 | A closer look at CPEB

To accomplish the tasks of repressing, activating and localizing mRNA, the cytoplasmic polyadenylation element (CPE)-binding protein (CPEB) associates with at least five proteins — Eg2, cleavage and polyadenylation specificity factor (CPSF), tubulin, maskin and pumilio[13,33,38,39,66,87], and, of course, mRNA. The functional structure of CPEB can be roughly divided into amino- and carboxy-terminal regions (a). The carboxy-terminal portion is devoted to RNA binding and contains two RNA-recognition motifs (RRMs; blue boxes) and a zinc finger (Zif, green box), all of which are necessary for optimal interaction with the CPE[34,88]. The amino-terminal portion of CPEB contains the regulatory information, such as a PEST (proline, glutamic acid, serine, threonine) box (red box) that mediates tubulin binding[66] and, possibly, proteasome-induced destruction[60]; the Eg2 phosphorylation site[38] (serine 174); and an Eg2-interacting element[38] (yellow box).



CPEB-like proteins are probably present in all metazoans. Whereas the carboxy-terminal portion is highly conserved among different animal groups, the amino-terminal portion varies considerably. For example, panel b shows a sequence compilation of metazoan CPEB-like proteins, in which the horizontal distance reflects the relative degree of divergence of the proteins from each other[89]. There are two main CPEB families, both of which contain the two RRM motifs and a $C_2C_2H_2$-type zinc finger. However, with the exception of *Drosophila melanogaster* Orb, the PEST box is present in all members of the 'classical' CPEB family. Although a PEST box is also detected in CPB-2, it is in a non-conserved position. In addition, only vertebrate CPEB proteins of both families contain obvious Eg2 phosphorylation sites.

Although the molecular functions of these multiple CPEB proteins have yet to be explored, their biological importance has been tested in *Caenorhabditis elegans*. Using RNA INTERFERENCE directed against each of the CPEB isoforms, Luitjens *et al.*[87] have shown that only CPB1 and FOG1 yield discernable phenotypes, which are defects in meiotic progression during spermatogenesis. Similarly, *Cpeb* null mice, both males and females, have meiotically defective germ cells that are arrested at pachytene. Moreover, two CPEB-associated mRNAs that encode components of the synaptonemal complex have shortened poly(A) tails and fail to be translated in the null mice[90].

Given that CPEB is so essential for *Drosophila* and vertebrate oogenesis, it is surprising that no oocyte phenotypes were observed in the worm. Perhaps CPEB does have a translational function in *C. elegans* oocytes, but it is dispensable for meiotic progression.

RNA INTERFERENCE (RNAi). A technique in which double-stranded RNA targeted against a gene product is introduced into cells or an organism, resulting in null or hypomorphic phenotypes.

PAM MATRIX
A matrix of weights derived from how often different amino acids replace other amino acids during evolution. PAM stands for 'per cent accepted mutations', and these were inferred from the types of change observed in these proteins. Every change was tabulated and entered in a matrix enumerating all possible amino-acid changes.

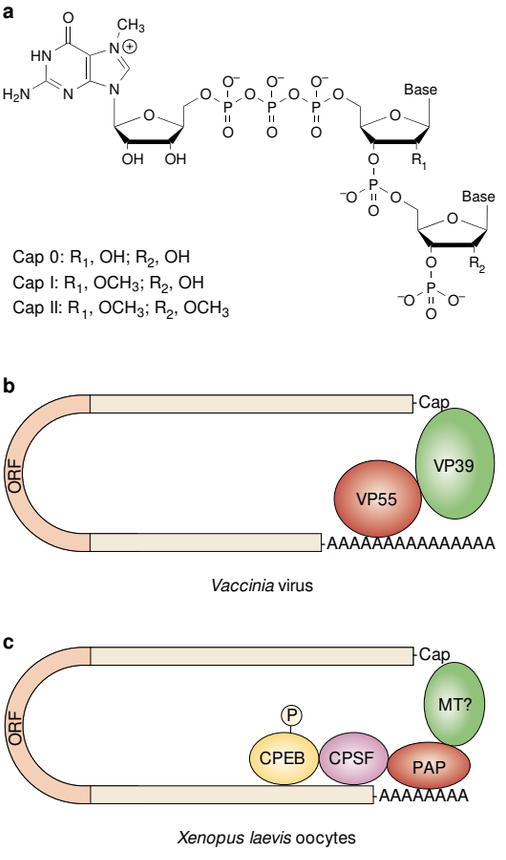


Figure 5 | **Cap-specific 2′-*O*-methylation. a** | Structure of the 5′ cap, denoting the 2′-*O*-methylations that distinguish cap 0 from cap I and cap II. **b** | Polyadenylation and cap methylation in *Vaccinia* virus. The VP55 subunit has polyadenylation activity, but this protein generates poly(A) tails that are only ~35 nucleotides in length. The VP39 subunit, which also has polyadenylation activity, is necessary for further elongation of the poly(A) tail. VP39 is a bifunctional enzyme because it also catalyses cap-specific 2′-*O*-methyaltion. **c** | Proposed model for polyadenylation-mediated cap-specific 2′-*O*-methylation in *Xenopus laevis* oocytes. When CPE-containing mRNAs are polyadenylated, their 5′ cap 0 structures are converted to cap I and cap II. The observation that continuing polyadenylation, but not a poly(A) tail *per se*, is necessary for cap methylation[48] indicates that poly(A) polymerase (PAP) might be involved in both 3′ and 5′ end modifications. Because *Xenopus* PAP has no detectable methyltransferase activity, a separate polypeptide probably catalyses this reaction. CPE, cytoplasmic polyadenylation element; CPEB, CPE-binding protein; CPSF, cleavage and polyadenylation specificity factor; MT?, unidentified methyltransferase.

maskin itself could all influence the assembly of the 48S initiation complex.

Another way polyadenylation induces translation in oocytes is by promoting cap-specific 2′-*O*-methylation (FIG. 5). The m^7GpppN cap structures on the 5′ ends of mRNAs are usually methylated on the base (N) or the ribose. As a consequence of continuing poly(A) elongation (as opposed to a static poly(A) tail), the cap 0 structure (lacking ribose methylation) on at least one mRNA is converted to cap I and cap II, which are distinguished by methyl groups on the first and second sugar moieties immediately downstream of the triphosphate bridge (FIG. 5a)[62]. Not only does abrogation of cap methylation inhibit translation, but an mRNA already containing a cap I structure is translated more efficiently than one containing a cap 0 after oocyte injection[62,63]. However, not all CPE-containing mRNAs undergo cap ribose methylation[64].

Although the mechanistic relationship between polyadenylation and cap ribose methylation is unknown, an instructive example is found in *Vaccinia* virus, in which the viral poly(A) polymerase and the methyltransferase activities reside in a single polypeptide (FIG. 5b)[65]. Although the oocyte poly(A) polymerase is unlikely to also have intrinsic methyltransferase activity, it is possible that polyadenylation and ribose methylation reactions are catalysed by two polypeptides that heterodimerize (FIG. 5c).

Polyadenylation, cell cycle and embryo polarity

Once polyadenylation takes place during oocyte maturation, most of the CPEB (~90%) is destroyed — virtually all that remains stable is confined to ANIMAL POLE BLASTOMERES, where it is strongly associated with spindles and centrosomes[66]. Maskin has a similar localization pattern. Not surprisingly, both proteins bind microtubules and, at least for CPEB, this interaction is a direct one, mediated by a small internal PEST (proline, glutamic acid, serine, theonine) domain. When injected into embryos, reagents that are known to disrupt polyadenylation-induced translation (for example, an antibody against CPEB, a CPEB dominant-negative mutant or 3′-DEOXYADENOSINE) inhibit cell division and produce abnormal mitotic structures, such as multiple centrosomes, centrosomes detached from spindles and tripolar spindles. These results indicate that embryonic cell division might require polyadenylation-induced translation, but they do not indicate where this requirement occurs (for example, soluble or spindle-associated), or what mRNA(s) might be involved.

Four observations pointed to cyclin B1 mRNA as the key molecule. First, it has a CPE and is regulated by cytoplasmic polyadenylation, at least in maturing oocytes[35]. Second, its translation is necessary for cell division[67,68]. Third, it is found on spindles in *Drosophila* embryos[69]. And finally, cyclin protein is found on spindles in HeLa cells[70]. Given this evidence, it is perhaps not surprising that cyclin B1 mRNA and protein were both found to be spindle-associated in *Xenopus* embryos. These data argue that cell division requires translation of cyclin B1 mRNA on spindles. Indeed, although the injection of a CPEB mutant protein lacking its microtubule-binding domain has little effect on cyclin B1 mRNA translation, it causes this message to dissociate from spindles. The consequence of this dissociation is the loss of cyclin B1 protein from spindles and, as a result, inhibited cell division. Therefore, CPEB controls not only cyclin mRNA translation but also its localization to spindles.

A similar picture of localized translational control emerges from the study of the *Drosophila* homologue

ANIMAL POLE BLASTOMERES
Embryonic cells that will form the ectoderm.

3′-DEOXYADENOSINE
Analogue of ATP that acts as a chain terminator during RNA synthesis or polyadenylation.

POSTSYNAPTIC DENSITY
Dense structure beneath the postsynaptic site, in which an array of synaptic proteins is anchored to a specific set of cytoskeletal and/or signalling proteins.

αCAMKII
Calcium–calmodulin-dependent kinase II; activated in response to synaptic activity.

of CPEB, Orb. Orb regulates the translation and localization of *oskar*[71,72] and *gurken*[71] mRNAs as well as *orb* mRNA itself[73]. This localized translational regulation is crucial for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis[71,74], as well as for formation of the egg chamber[74] and entry into meiosis[75]. Although not as well characterized, the zebrafish homologue of CPEB, Zorba[76], is also localized to the dorsal part of the embryo; however, the significance of this observation remains to be determined. In *Xenopus* embryos, at least one mRNA, encoding Xwnt-11, undergoes cytoplasmic polyadenylation in a dorsal compartment[77], perhaps suggesting a conserved mechanism for the formation of body pattern in vertebrates.

Polyadenylation and synaptic plasticity

Although polyadenylation-induced translation is a characteristic of early development in probably all metazoans[28,29,51,52,78,79], one question is whether this type of regulation is restricted to early development. Biochemical demonstration of cytoplasmic polyadenylation in somatic cells is difficult, but the presence of CPEB would certainly suggest that this is a distinct possibility. Early studies in the mouse did not reveal significant levels of CPEB outside the ovary and testis[80], but more recent analysis[81] showed that it is moderately prevalent in the brain. Further investigation showed[81] CPEB to be present in the hippocampus, at synapses of cultured hippocampal neurons, and to co-fractionate with the POSTSYNAPTIC DENSITY fraction. This localization pattern is potentially important because synaptic plasticity is controlled, at least in part, by the translation of mRNAs stored in dendrites[82–86]. Indeed, the stimulation of synapses induces the polyadenylation and translation of a CPE-containing mRNA in dendrites (encoding calcium–calmodulin-dependent kinase II (αCAMKII)), but not of an mRNA that lacks a CPE (neurofilament)[81]. These results imply that CPEB-controlled translation might influence synaptic plasticity and, possibly, long-term memory.

The roads ahead

The delineation of the core features of cytoplasmic polyadenylation leads us to suggest that two broad avenues of research lie ahead. The first one is to understand more about the mechanism of this process. In *Xenopus* oocytes, it is clear that there is more specificity to mRNA translation by cytoplasmic polyadenylation than can be accounted for by the mere presence or absence of a CPE. Recall that Mos activity seems to be necessary for the polyadenylation of mRNAs with several CPEs. We do not yet understand the relationship between CPE copy number or the sequences adjacent to the CPE and Mos dependency for polyadenylation. Another important mechanism to decipher is the regulation of maskin–eIF4E binding. Does their dissociation require polyadenylation (and, by extension, the poly(A)-binding protein)? If not, what processes determine when maskin and eIF4E dissociate in an mRNA-specific manner? Furthermore, how are the three CPE-mediated events (mRNA repression, activation and localization) coordinately regulated during the embryonic cell cycle?

The second avenue of pursuit is biological. By any measure, the possible involvement of CPEB and polyadenylation in cognitive function is an exciting prospect, and experiments that address translational control in the central nervous system, by any mechanism, will be eagerly anticipated. Finally, what is the purpose of multiple CPEB proteins, especially in mammals? Do they all support polyadenylation, or do they have diverse functions as indicated by the experiments in *Caenorhabditis elegans*? Are different mRNAs targeted by different CPEB isoforms? One intriguing possibility is that CPEB isoforms control translation in a tissue-specific manner in response to different external stimuli. Certainly, several approaches with different animal models will help answer these questions.

Links

DATABASE LINKS eIF4E | eIF4A | eIF4G | PABP | eIF3 | eIF2 | IRP | Mos | MAPK | cyclin B | p90^{rsk} | cdc2 | CPEB | CPSF | poly(A) polymerase | Orb | *oskar* | *gurken* | Zorba

FURTHER INFORMATION Richter lab

ENCYCLOPEDIA OF LIFE SCIENCES Translation control by RNA | Translation initiation models in prokaryotes and eukaryotes

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