Regulation of mRNA translation by 5′- and 3′-UTR-binding factors☆

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The translational regulation of specific mRNAs is important for controlling gene expression. The past few years have seen a rapid expansion in the identification and characterization of mRNA regulatory elements and their binding proteins. For the majority of these examples, the mechanism by which translational regulation is achieved is not well understood. Nevertheless, detailed analyses of a few examples show that almost every event in the initiation pathway, from binding of the cap complex to the joining of the 60S ribosomal subunit, is subject to regulation.

Translational control of specific mRNAs is a widespread mechanism of gene regulation and contributes to diverse biological processes in many cell types. A large number of regulatory elements and their binding proteins have been identified, and their characterization is leading to a greater understanding of the mechanisms of translational control. In some cases, the biological consequences of mis-regulation are also becoming clear. This review focuses on the mechanisms by which RNA elements and their interacting factors regulate translation, highlighting a few examples from animals and referring to yeast, plant and viral mRNAs as needed.

Although many features of an mRNA can contribute to its translation, most control elements are located within the untranslated regions (UTRs; Fig. 1). The 5′ m^7GpppG cap and the 3′ poly(A) tail are important determinants of translational efficiency. Overall translation rates are also affected by characteristics of the 5′ UTR, including length and start-site consensus sequences as well as the presence of secondary structure, upstream AUGs, upstream open reading frames (uORFs) and internal ribosome entry sites (IRES) [1,2]. In addition, 5′ UTRs can contain sequences that function as binding sites for regulatory proteins. Similarly, 3′ UTRs contain numerous binding sites for regulatory factors [1–3]; these factors are usually proteins, but in a few cases *trans*-acting RNAs have been described. Most, but not all, of the elements described affect translation at the level of initiation.

Translation initiation of most eukaryotic cellular mRNAs can be divided into three basic mRNA-dependent steps: (1) the small (40S) ribosomal subunit binds to the mRNA at or near the m^7GpppG cap – an interaction that is aided by several initiation factors (eIFs); (2) the small ribosomal subunit and associated factors scan through the 5′ UTR to the initiation codon; and (3) initiation factors are released and the large (60S) ribosomal subunit joins, forming an 80S ribosome that is competent to begin elongation. A more detailed description of initiation, including the role of eIFs, is shown in Fig. 2 (for a review see [4,5]).

Repression by 5′-UTR-binding proteins – iron regulatory protein

Much of our understanding about the function of 5′-UTR repressor proteins comes from detailed analyses of a single RNA–protein complex. Iron regulatory proteins (IRPs) control several mRNAs that contain a stem–loop structure known as the iron-responsive element (IRE), in response to intracellular iron concentrations. The importance of this RNA–protein complex is highlighted by a genetic disorder caused by mutations within the IRE [6].

In most cases, IREs are located close to the m^7GpppG cap; analyses indicate that this position is important for their regulatory mechanism [1]. Sucrose gradient analyses of initiation intermediates have shown that cap-proximal IRE–IRP complexes sterically inhibit the binding of 40S ribosomal subunits to mRNA [1]. However, these complexes do not impede the interaction of initiation factors that bind before the 40S ribosomal subunit [7]. Importantly, when IRE–IRP complexes are located in a cap-distal position they no longer affect 40S recruitment, but cause a degree of inhibition by impeding scanning [8]. Thus, one RNA–protein complex can inhibit two different steps of initiation depending on its position within the 5′ UTR. Moreover, IRE–IRP-type regulation can be observed when exogenous RNA–protein complexes, which do not normally mediate translational control, are placed within the 5′ UTR [1]. This suggests that IRE–IRP regulation can provide a framework for other 5′-UTR-repressor proteins. In support of this idea, auto-regulation of poly(A)-binding protein (PABP) mRNA was recently shown to exhibit similarities with that of cap-distal IRP complexes. PABP binds to a cap-distal poly(A) tract in its own 5′ UTR and represses translation [9,10]. Analysis of PABP mRNA using sucrose gradients is consistent with the model that PABP inhibits scanning of the 40S ribosomal subunit [10]. Although it is attractive, the IRE–IRP model of regulation can not necessarily be applied to all 5′-UTR regulatory mechanisms. For instance, mRNAs containing a 5′-terminal oligopyrimidine tract (TOP) show extreme position

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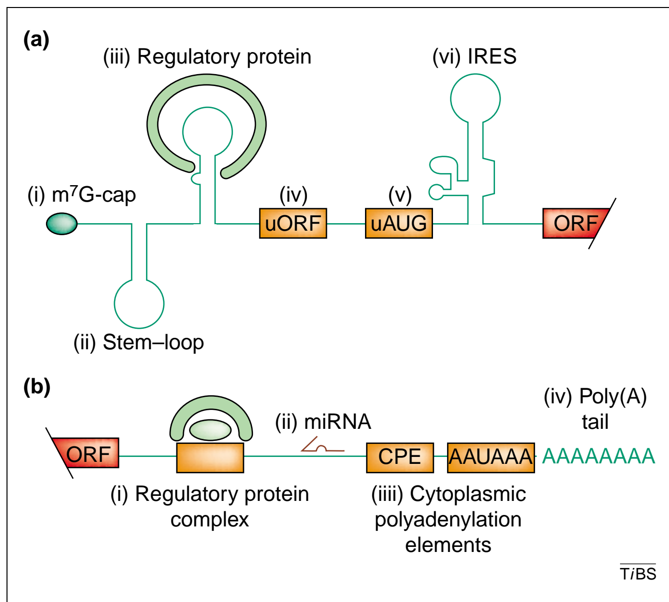

Fig. 1. Elements within untranslated regions (UTRs) that regulate translation. The open reading frame (ORF; red) denotes the main ORF. (a) 5′ UTRs contain diverse regulatory elements. (i) The m^{7}GpppG cap (dark green) is a crucial determinant of translational efficiency because it is recognized by the cap-binding complex eIF4F. (ii) Secondary structure, or stem–loops, negatively affect translation by impeding the binding or migration of 40S ribosomal subunits. (iii) Regulatory proteins interact with specific elements within the 5′ UTR, these elements are often structured. 5′-UTR-interacting proteins often repress translation and do so in a manner analogous to secondary structures. (iv) Upstream ORFs (uORF) and (v) upstream AUGs (uAUG) normally down-regulate translation at the main ORF by providing alternative start sites. uAUGs must be in a different frame to the main ORF. Often uORFs are less inhibitory than uAUGs because ribosomes have the potential to re-initiate translation at the main ORF following termination at the stop codon of the uORF. (vi) Internal ribosome entry sites (IRES) promote cap-independent initiation, an alternate form of initiation in which ribosomal subunits are recruited to the IRES either directly or by a subset of initiation factors. (b) 3′ UTRs can also contain a multitude of translational regulatory elements. (i) Elements can act as recognition sites for regulatory proteins (green). These elements can be structured or unstructured. Often, 3′-UTR regulation requires a complex of regulatory proteins rather than a single protein. (ii) Short 21-nucleotide anti-sense microRNAs (miRNAs) repress translation function by targeting complimentary sequences within the 3′UTR. (iii) Cytoplasmic polyadenylation elements (CPE) and the hexanucleotide AAUAAA are required to activate poly(A)-tail lengthening of an mRNA. In addition, CPEs have been suggested to play a role in translational repression. (iv) The poly(A) tail plays an important role in translation. Increases in poly(A)-tail length stimulate translation and might do so by recruiting additional poly(A)-binding protein (PABP) molecules.

dependence – the TOP element can not even be moved by one nucleotide [11], a characteristic not shared by the IRE–IRP system.

As cap-proximal complexes might regulate translation more efficiently, the question of why some 5′ UTRs contain cap-distal versus cap-proximal complexes is raised. In some cases, this difference could reflect a requirement to down-regulate rather than effectively switch off translation. The use of different transcription start sites that alter the distance between the cap and the regulatory complex, alongside the ability of different cell types to more efficiently overcome some cap-distal complexes [1], could enable exquisite control of an mRNA. Therefore, it is surprising that more 5′-UTR regulatory proteins have not been conclusively identified, and that 3′-UTR regulatory complexes appear to be more common despite the complexity of their action.

Regulation by 3′-UTR-binding factors

Unlike the studied cases of 5′-UTR regulation, mechanistic analyses of 3′-UTR regulation have proved conceptually more difficult. In recent years, several models have been proposed to explain how complexes at the 3′ end of the mRNA might affect translation [1,3,12] and, in a few cases, molecular mechanisms are beginning to emerge.

Translational activation via PABP

Changes in the translation of mRNAs are frequently correlated with cytoplasmic changes in poly(A)-tail length; increases in length generally correlate with translational activation. This phenomenon has been widely studied during early development in higher eukaryotes [3], but has also been reported in somatic cells. For example, at least one dendritic mRNA is thought to be regulated by changes in poly(A)-tail length [13]. However, the mechanism by which poly(A) tails control translation is not fully understood.

The poly(A) tail and m^{7}GpppG cap are located at opposite ends of the mRNA molecule, but act synergistically to stimulate translation [14,15]. Consequently, popular models for how 3′ poly(A) tails might influence initiation at the 5′ end generally focus on factors associated with mRNA termini. Interactions between these factors might form end-to-end complexes, effectively circularizing the mRNA. In support of this, mRNAs have been visualized as circular structures by microscopy [16,17]. A protein suggested to play a crucial role in poly(A)-mediated translation is PABP – a multifunctional protein with roles in mRNA processing, stability and translation [15]. Interestingly, several translation factors interact with PABP, and some of

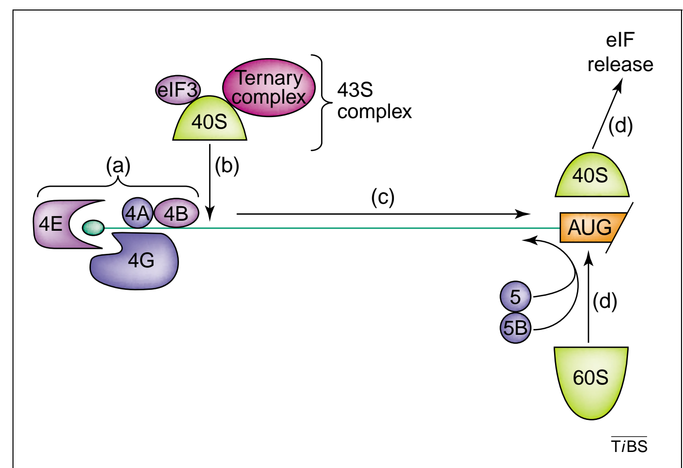

Fig. 2. The cap-dependent initiation pathway. (a) Eukaryotic initiation factor eIF4E (4E) binds to the m^{7}GpppG cap (green) as part of a protein complex known as eIF4F. In animal cells, this complex also contains eIF4G (4G), a central scaffolding protein for other initiation factors, and eIF4A (4A), an RNA-dependent helicase. eIF4B (4B) is also recruited to the mRNA and appears to stimulate eIF4A helicase activity, which is thought to unwind secondary structure within the 5′ UTR. (b) The 43S pre-initiation complex, containing the small (40S) ribosomal subunit, the ternary complex (initiator Met-tRNA$_i$–eIF2–GTP) and additional initiation factors, then binds at or near the m^{7}GpppG cap. This requires several initiation factors; a direct interaction between eIF3 (located on the small ribosomal subunit) and eIF4G (part of the eIF4F cap-binding complex) is thought to be pivotal for this recruitment. (c) The 43S pre-initiation complex, aided by associated factors, migrates to the initiation codon, which is normally the first AUG encountered, in a process often referred to as scanning. (d) Finally, initiation factors are released and the large (60S) ribosomal subunit joins to form an 80S ribosome. This final step is GTP-dependent and requires the activities of eIF5 (5) and eIF5B (5B). The role of several initiation factors is not depicted, see [4,5] for a more detailed description. The figure is schematic and is not meant to indicate the spatial arrangement of proteins within the various complexes nor the full extent of RNA–protein or protein–protein interactions.

these might play an important role in mediating end-to-end complexes (Fig. 3).

The interaction between PABP and eIF4G has been extensively studied. This interaction can be detected in a wide range of species including yeast, plants and vertebrates [1,14], and would bring the ends of the mRNA into proximity owing to the association of eIF4G with the cap-binding protein eIF4E. Evidence from several sources suggests that the PABP–eIF4G interaction is important for translational regulation. For example, interference with PABP–eIF4G interactions via truncation of eIF4G or overexpression of mutant eIF4G results in decreased poly(A)-mediated translation [18,19]. Furthermore, expression of a PABP fragment that interacts with eIF4G is sufficient to stimulate translation of reporter mRNAs in *Xenopus laevis* when artificially brought to the mRNA [20]. The mechanism by which the PABP–eIF4G interaction might regulate translation has been suggested to involve the stabilization of poly(A)–PABP interactions [21,22] and/or an increase in the affinity of eIF4F for the m^{7}GpppG cap [23–25]. Stabilizing the end-to-end complex could enhance translation by recruitment of 40S ribosomal subunits through eIF4G–eIF3 interactions (Fig. 2 legend).

Although interaction with eIF4G might be important, other evidence suggests that poly(A)–PABP-mediated translation can occur via additional mechanisms. Mutations in eIF4G that eliminate binding to PABP do not disrupt the viability or growth rate of yeast cells, and a poly(A) tail is still required for efficient translation in these strains [18,26]. Furthermore, a fragment of *X. laevis* PABP that lacks the eIF4G-interaction site can stimulate the translation of reporter mRNAs as effectively as full-length PABP [20]. In light of this, it is interesting to note that several other translation factors interact with PABP.

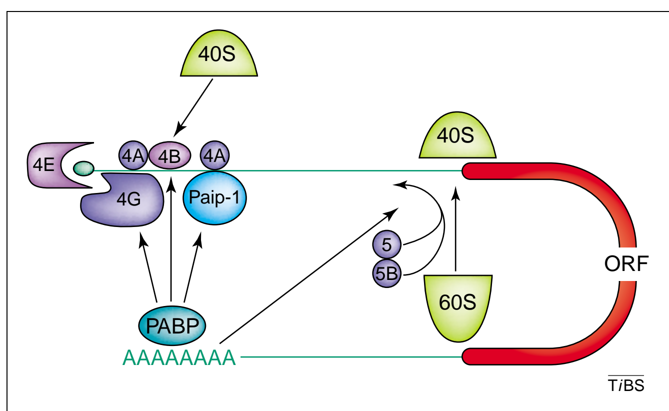

Fig. 3. Regulation of mRNAs by poly(A) tails. In general, long poly(A) tails lead to translational activation, whereas short poly(A) tails do not. The affect of the poly(A) tail is thought to be mediated by poly(A)-binding protein (PABP). PABP physically interacts with eukaryotic initiation factor eIF4G (4G), Paip-1 and eIF4B (4B). The PABP–eIF4G interaction is proposed to circularize the mRNA via PABP–eIF4G–eIF4E–cap interactions. Paip-1 has similarities to eIF4G and interacts with eIF4A, but not eIF4E, suggesting that it might stimulate translation by a different end-to-end mechanism. The PABP–eIF4B interaction has been suggested to enhance PABP binding to poly(A), and to stimulate the activity of the eIF4A helicase. All of these interactions are predicted to affect the recruitment of the small (40S) ribosomal subunit to the mRNA. Genetic evidence in yeast suggests a connection between poly(A) tails and eIF5B. Poly(A) tails have been suggested to have an indirect effect on the activity of eIF5B, and this is thought to affect 60S-ribosomal-subunit joining. The figure is schematic and is not meant to indicate the spatial arrangement of proteins within the various complexes nor the full extent of RNA–protein or protein–protein interactions.

In mammalian cells, a PABP-interacting protein (Paip-1) that shares considerable homology with the C terminus of eIF4G has been identified [27]. Overexpression of Paip-1 in mammalian cell lines causes a modest increase in the translation of reporter mRNAs [27]. A PABP–Paip-1 interaction is also detectable in *X. laevis*; however, the Paip-1 interaction domains of *X. laevis* PABP are not sufficient to promote translation in oocytes, casting doubt over a dominant role of Paip-1 in translation in these cells [20]. Interestingly, a multi-protein complex including PABP and Paip-1 has been shown to affect the stability of c-*fos* mRNA in mammalian cells, suggesting an alternative function for Paip-1 [28].

PABP also interacts with eIF4B, an initiation factor that aids the processivity of the eIF4A RNA helicase [4,5]. This interaction was initially detected in plants [21], and has been suggested to enhance both poly(A)–PABP binding and eIF4A–eIF4B helicase activity [21,29]; the latter might promote removal of 5′-UTR secondary structure. The interaction of mammalian PABP with eIF4B is disrupted by some viral proteases and by apoptosis, suggesting an important physiological role [30]. However, the relative contribution of this interaction to poly(A)-mediated translation remains to be determined.

PABP also binds to eukaryotic release factor 3 (eRF3 or GSPT) [31,32]. This interaction could provide support for the model that poly(A) tails promote the recycling of terminating ribosomes from the 3′ to the 5′ end of mRNA [14,15], but a functional role for this interaction in poly(A)-mediated translation remains to be conclusively demonstrated.

Furthermore, recent genetic evidence in yeast suggests that poly(A) tails might function indirectly by affecting the activity of eIF5B [26], an initiation factor involved in 60S-ribosomal-subunit joining. However, further work is required to clarify the putative links between eIF5B and poly(A) function, and whether this effect involves PABP.

Several PABP-interacting proteins have now been identified, and these function at multiple steps in the initiation pathway. However, which of these interactions are physiologically relevant remains to be determined. Moreover, none of the models adequately explain how changes in poly(A)-tail length alter translation. A popular idea is that increases in poly(A)-tail length might result in recruitment of additional PABP molecules. This leads to the question of how many interactions a single molecule of PABP can make, and whether the binding of different partners to PABP is sequential.

Translational repression via cytoplasmic polyadenylation-element-binding protein

Cytoplasmic poly(A)-tail length can be regulated by elements within the 3′ UTR. The poly(A) tail appears to act through PABP (as discussed). However, short poly(A) tails are often long enough to bind PABP, yet messages can remain translationally silent [1]. This suggests that some mRNAs are specifically maintained in an inactive state, potentially via binding of repressor proteins. The recent identification of one 3′-UTR-binding protein, cytoplasmic polyadenylation-element-binding protein (CPEB), has

provided clues to how polyadenylation and translational repression are linked [33].

Cytoplasmic polyadenylation requires two 3′-UTR elements: a uridine-rich sequence known as a cytoplasmic polyadenylation element (CPE) or adenylate control element (ACE) and the hexanucleotide AAUAAA. Three proteins – CPEB, cleavage and polyadenylation-specificity factor (CPSF) and a poly(A) polymerase – are also required. Regulated phosphorylation of CPEB appears to be a key mechanism in the activation of this event and has been discussed in depth elsewhere [33].

Interestingly, some CPEs have also been suggested to repress translation before directing adenylation [34–37] and, recently, a mechanism for this has been proposed [38]. CPEB appears to mediate these repressive effects in *X. laevis* oocytes, clams and mice [34–36]. These observations suggest that CPEB performs dual roles in translation – one in mRNA repression and one in activation – possibly by changing the protein partners with which it interacts. Repression of certain CPE-containing mRNAs in *X. laevis* also appears to require maskin, a protein that interacts with both CPEB and eIF4E [38]. The interaction of maskin with eIF4E is via an eIF4G-like domain that resembles those present in 4E-binding proteins (4E-BPs), factors known to globally block cap-dependent translation by sequestration of eIF4E [39]. This observation provides a model for CPEB-mediated repression in which CPEB and eIF4E are bridged by maskin, making them unavailable for interaction with other proteins. In support of this, the maskin eIF4G-like peptide inhibits translation when injected into *X. laevis* oocytes [38] in a manner analogous to that of 4E-BPs.

Recent data directly links poly(A)-mediated activation to loss of CPEB–maskin-mediated repression. CPEB can only repress mRNAs containing poly(A) tails under a critical length [33]. Furthermore, the maskin–eIF4E interaction weakens during meiotic maturation, when poly(A)-mediated translational activation is observed [33,40]. A model to explain these findings has been proposed: PABP, bound to the extended poly(A) tail, interacts with eIF4G effectively competing with maskin for eIF4E binding [40].

Although these results are thought provoking, several questions remain. First, CPE-mediated repression occurs in early- as well as late-stage oocytes; maskin is present in the late-stage oocytes of both *X. laevis* and mice [38,41], but it is not present in immature oocytes [42]. Second, only some CPE-containing mRNAs are repressed [43,44]; as CPEB recognizes all studied CPEs, maskin should likewise be recruited to these mRNAs, resulting in their translational repression. This implies that the CPE–CPEB–maskin complex alone is insufficient to mediate repression, and that other sequence-specific binding factors are likely to be involved.

A recent report provides preliminary clues to the identity of another potential player – a member of the Pumilio/Fem3-binding protein (PUF) family; PUF proteins play roles in translational repression in both *Drosophila* and *Caenorhabditis elegans* [45]. Recently, an *X. laevis* homologue of Pumilio (XPum), which is present throughout oogenesis, was shown to interact with CPEB, but its role in translational repression remains unclear. Interestingly, in *C. elegans*, Fem3-binding protein interacts with a CPEB homologue [46], suggesting that CPEB–PUF interactions might play a general role in CPEB-mediated translational control.

Repression of 15-lipoxygenase mRNA

Not all regulatory mechanisms that operate through the 3′ UTR require changes in poly(A)-tail length. In some cases, changes in poly(A)-tail length occur as a consequence, rather than a cause, of translational regulation. 15-lipoxygenase (LOX) mRNA encodes a protein that mediates mitochondrial breakdown during the terminal stages of erythrocyte differentiation. In erythroid precursor cells, the translation of LOX mRNA is repressed by the binding of heterogeneous nuclear (hn) ribonucleoprotein (RNP) K and hnRNP E1 to a differentiation control element (DICE) in the LOX 3′ UTR [47]; in the case of hnRNP K, this involves regulated phosphorylation [48]. DICE-mediated repression is independent of poly(A)-tail status *in vitro* [1], suggesting that hnRNP K and hnRNP E1 do not interfere with the function of the poly(A) tail.

To determine the step during initiation in which hnRNP K and hnRNP E1 function, the ability of DICE elements to impose regulation on reporter mRNAs containing unusual IRES elements has been tested. These IRES elements do not require certain canonical initiation factors, allowing factors that are not required for DICE regulation to be eliminated. Sucrose gradient analyses of initiation intermediates were also performed. Together, these experiments suggest that, in contrast to IRP-mediated translational repression, hnRNP K and hnRNP E1 do not interfere with the joining or transit of 40S ribosomal subunits. Toe-printing analyses have confirmed that 40S ribosomal subunits were present at the AUG. As 80S ribosomes were not present on these repressed messages, it was suggested that hnRNP K and hnRNP E1 impair the joining of the 60S ribosomal subunit [49].

It is currently unclear exactly how 60S-ribosomal-subunit joining is impaired. But it has been proposed that an initiation factor involved in 60S joining might be targeted directly or indirectly by hnRNP K or hnRNP E1 [49]. An interesting, and as yet unanswered, question is the identity of target translation factor(s) and the mechanism by which interactions between these factors and the repressive complex actually lead to inhibition.

Regulation of *oskar*-linking mRNA localization to translation

It is clear that many mRNAs are localized within the cytoplasm and that localization is intimately linked to translational regulation. This is exemplified by studies of *oskar* (*osk*) mRNA. Osk protein is restricted to the posterior of *Drosophila* oocytes and embryos, where it is required for abdominal patterning and germ-cell formation. *osk* mRNA is synthesized in nurse cells and transported into the oocyte, where it becomes specifically localized to the posterior pole (Fig. 4). To ensure *osk* is not ectopically expressed, unlocalized *osk* mRNA is repressed and activated only when the mRNA is correctly localized.

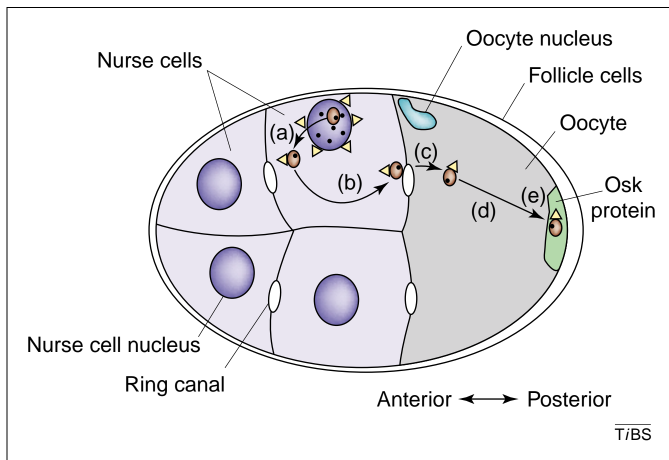

Fig. 4. Localized translation ensures that synthesis of Oskar protein (green) occurs exclusively at the posterior of a *Drosophila* oocyte. A stage-nine egg chamber is represented, but only four of the 15 nurse cells are depicted for simplicity. The oocyte nucleus is thought to be largely transcriptionally inactive, and the nurse cells support the oocyte by synthesizing macromolecules required for growth and development. These are transported into the growing oocyte through a network of ring canals, specialized cell–cell junctions that interconnect the nurse cells and oocyte. (a) *oskar* (*osk*) is transcribed in nurse cell nuclei. During splicing, *osk* mRNA (brown circles) associates with proteins later required for its cytoplasmic localization (small black circles), including Mago nashi and Y14/Tsunagi [70]. After export to the cytoplasm, the *osk* mRNP complex recruits additional proteins required for its transport and translational repression such as Exuperantia, Barentsz, Ypsilon Schachtel and Me31B. Many of these proteins (yellow triangles) are concentrated in the cytoplasm surrounding each nurse cell nucleus. This might facilitate their binding to newly-exported mRNAs. (b) The *osk* mRNP complex is transported through the nurse-cell cytoplasm to the ring canal in a microtubule-dependent manner. Me31B is required to maintain translational repression during this stage. (c) This complex is then transported through the ring canal into the oocyte, a step that is independent of both the microtubule and actin cytoskeleton. (d) The molecular motor kinesin is then required to drive the transport of *osk* mRNP particles to the plus ends of microtubules at the posterior of the oocyte. Localization also requires Staufen, Exuperantia and Barentsz [71,72]. During transport, translational repression is maintained by Bruno, p50, Bicaudal-C and Apontic. (e) Once anchored at the posterior pole translation of *osk* mRNA ensues. Full translational activation requires Aubergine, Vasa, p50, Staufen, Orb and Oskar protein itself.

Many of the factors involved in this regulation have been isolated by genetic and biochemical means.

Repression of *osk* mRNA during transport from nurse cells to the oocyte requires Me31B, a DEAD-box protein [50], whereas the RNA-binding protein Bruno is essential for the repression of unlocalized *osk* in the oocyte. Bruno interacts with elements in the 3′ UTR of *osk* mRNA, although complete repression also requires p50, Bicaudal-C and Apontic [51]. The availability of oocyte *in vitro* translation extracts has started to reveal clues as to the mechanism of repression by Bruno [52,53]; *in vitro* repression of *osk* mRNA by Bruno does not require a poly(A) tail. Furthermore, repression is independent of the m^7GpppG cap, indicating that Bruno targets a step of translation that is downstream of the initial cap-binding event. More work is required to determine which of the subsequent events in translation is sensitive to Bruno.

In addition to the relief of Bruno-mediated repression, full activation of *osk* mRNA requires the action of several genes including *aubergine, vasa, staufen* and *orb* [51]. Interestingly, both *aubergine* and *vasa* encode proteins that resemble translation initiation factors. Vasa is a relative of eIF4A, but bears most similarity to Ded1p – a DEAD-box RNA helicase required for translation initiation in yeast [54,55]. Importantly, Vasa interacts with and might recruit IF2/eIF5B [56], a factor required for 60S-ribosomal-subunit joining in higher eukaryotes [57]. Therefore, Vasa might stimulate *osk* translation by promoting 60S recruitment, although this remains to be tested.

Aubergine is related to eIF2C, and is recruited to the posterior pole where it might function to promote the translation of a subset of mRNAs including *osk* [58]. Although the role of eIF2C in translation is poorly defined, it was first isolated as a protein that stabilizes the Met-tRNA$_i$–eIF2–GTP ternary complex. A clearer understanding of the role of eIF2C in initiation might be necessary to appreciate the role of Aubergine in *oskar* translation. Synthesis of Osk protein also requires Orb, the *Drosophila* homologue of *X. laevis* CPEB. Orb has been suggested to maintain or lengthen the poly(A) tail of *osk* mRNA *in vivo* [59]. Therefore, although Bruno-mediated repression does not require a poly(A) tail [52,53], polyadenylation might play a role in the activation of *osk* mRNA at the posterior. Analysis of Ypsilon Schachtel (Yps), a *Drosophila* Y-box protein, has provided further insights into the role of *orb*. Yps is found in a large mRNP complex containing Exuperantia, Me31B and *osk* mRNA [50,60]. Yps also interacts specifically with Orb protein in an RNA-dependent manner [61]; genetic evidence suggests that Yps might act as a repressor by antagonizing the action of Orb during *osk*-mRNA transport, preventing the polyadenylation of *osk* mRNA until it is correctly localized [61].

Future work is required to identify the mechanisms by which these and other factors achieve the precise translational regulation of *osk* and other mRNAs that are essential for axis specification. Furthermore, it will be interesting to determine whether these proteins have evolved specific functions to control the expression of a few key transcripts during early development, or whether they play a wider role in translation.

Translational silencing by micro RNAs

The other regulatory events discussed here involve RNA–protein interactions. In *C. elegans*, a few cases have been described in which the *trans*-acting regulator is actually a small RNA, dubbed microRNA (miRNA). Recent reports demonstrate the presence of numerous miRNAs in *C. elegans* [62,63], *Drosophila* and humans [64]; the identified miRNAs exhibit both cell-type and stage-specific expression [62,64] suggesting that miRNA-mediated regulation could be widespread.

Only three cases of miRNA-mediated repression have been characterized to date, all of which are involved in developmental timing in *C. elegans*. The *lin-4* miRNA binds to both *lin-14* and *lin-28* 3′ UTRs, and the *let-7* miRNA interacts with the *lin-41* 3′ UTR [1,65]. In the case of the *lin-4*–*lin-14* interaction, the formation of a specific-bulged cytosine nucleotide appears crucial to the repression mechanism [1,65]. Although the mechanism by which these miRNAs repress translation is unknown, the presence of bulged nucleotides has been taken as potential evidence for the requirement of accessory proteins; this is an attractive model, but proteins that interact with these RNA duplexes are yet to be identified. Alternatively, bulged nucleotides might serve a function in preventing RNA-interference mediated effects. In support of this idea,

a recent report has shown that an miRNA–reporter-mRNA duplex containing bulged nucleotides represses translation *in vivo* and that exact complimentarity results in mRNA degradation [66].

The identification of multiple miRNAs illustrates the power of the 'genomic age': initial isolation of miRNAs was achieved by size fractionation, but subsequent validation was made possible by the availability of genome databases. Computational methodologies have enabled confirmation that miRNAs are not degraded mRNAs or small structural RNAs, but are encoded within intragenic regions. In some instances, evolutionary conservation is indicated, which strengthens arguments that miRNAs play important biological roles. Computational analyses have also identified potential targets for some human miRNAs [67]. Interestingly, these target mRNA sequences (K and Brd boxes) have been suggested to play roles in mRNA destabilization and translational repression [68,69]. Although at present the extent to which miRNAs impact cellular processes remains unknown, the sheer abundance of miRNAs suggests that this regulatory mechanism could be extensive.

Concluding remarks and future perspectives

It is now clear that multiple steps in the initiation pathway are targeted by different regulatory proteins. Furthermore, insights into how sequences within the 3′ UTR modulate translation have been achieved. One aspect that has emerged is the importance of close functional links between the 5′ and 3′ UTRs. The models outlined here, although not comprehensive, form a framework for understanding the function of other regulatory protein complexes.

Recent work has highlighted the complexity of regulatory elements present within mRNAs. In many cases, mRNAs contain multiple elements that can affect the final production of protein. However, knowledge of how these elements function within the context of the whole mRNA to achieve overall regulation is often lacking. This highlights the importance of relating powerful mechanistic studies of isolated elements to the regulation of endogenous mRNAs. An additional complexity is that elements within the same mRNA are likely to undergo differential regulation dependent upon environment, such as cell-type or spatial and/or temporal contexts.

It is also evident that translational control is closely associated with other mechanisms of gene regulation; there are close links between translation, mRNA stability and intracellular localization. Although many of the key factors linking these processes have been identified, a detailed understanding of their interactions and mechanisms has yet to emerge. Intriguingly, regulation in the cytoplasm is also related to events in the nucleus, and an increasing number of regulatory factors appear to influence events in both compartments. Understanding these links is clearly an important goal.

This review has dealt with a few specific examples of translational control that are well understood, but an increasing number of regulated mRNAs are being described. These mRNAs are involved in diverse biological functions, and occur in many different cell types and in many different organisms. The importance of UTRs in the regulation of gene expression is further underlined by the increase in reported diseases caused by mutations linked to translation [6]. Future work will be required to fully understand the mechanisms and biological importance of these regulatory RNA–protein complexes.

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References

1 Gray, N.K. and Wickens, M.P. (1998) Control of translation initiation in animals. *Annu. Rev. Cell Dev. Biol.* 14, 399–458
2 Mignone, F. *et al.* (2002) Untranslated regions of mRNAs. *Genome Biol.* 3, 4
3 Wickens, M. *et al.* (1997) Life and death in the cytoplasm: messages from the 3′ end. *Curr. Opin. Genet. Dev.* 7, 220–232
4 Hershey, J.W.B. and Merrick, W.C. (2000) Pathway and mechanism of initiation of protein synthesis. In *Translational Control of Gene Expression* (Sonenberg, N. *et al.*, eds), pp. 33–88, Cold Spring Harbour Laboratory Press
5 Dever, T.E. (2002) Gene-specific regulation by general translation factors. *Cell* 108, 545–556
6 Cazzola, M. and Skoda, R.C. (2000) Translational pathophysiology: a novel molecular mechanism of human disease. *Blood* 95, 3280–3288
7 Muckenthaler, M. *et al.* (1998) IRP-1 binding to ferritin mRNA disrupts the bridging interaction between cap-binding complex and the small ribosomal subunit. *Mol. Cell* 2, 383–388
8 Paraskeva, E. *et al.* (1999) Ribosomal pausing and scanning arrest as mechanisms of translational regulation from cap-distal iron-responsive elements. *Mol. Cell. Biol.* 19, 807–816
9 De-Melo-Neto, O.P. *et al.* (1995) Autoregulation of poly(A)-binding protein synthesis *in vitro*. *Nucleic Acids Res.* 23, 2198–2205
10 Bag, J. (2001) Feedback inhibition of poly(A)-binding protein mRNA translation. A possible mechanism of translation arrest by stalled 40 S ribosomal subunits. *J. Biol. Chem.* 276, 47352–47360
11 Meyuhas, O. (2000) Synthesis of the translational apparatus is regulated at the translational level. *Eur. J. Biochem.* 267, 6321–6330
12 Preiss, T. and Hentze, M.W. (1999) From factors to mechanisms: translation and translational control in eukaryotes. *Curr. Opin. Genet. Dev.* 9, 515–521
13 Jiang, C. and Schuman, E.M. (2002) Regulation and function of local protein synthesis in neuronal dendrites. *Trends Biochem. Sci.* 27, 506–513
14 Gallie, D.R. (1998) A tale of two termini: a functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation. *Gene* 216, 1–11
15 Jacobson, A. (1996) Poly(A) metabolism and translation: the closed-loop model. In *Translational Control* (Hershey, J. *et al.*, eds), Cold Spring Harbor Laboratory Press
16 Christensen, A.K. *et al.* (1987) Circular polysomes predominate on the rough endoplasmic reticulum of somatotropes and mammotropes in the rat anterior pituitary. *Am. J. Anat.* 178, 1–10
17 Wells, S.E. *et al.* (1998) Circularization of mRNA by eukaryotic translation initiation factors. *Mol. Cell* 2, 135–140
18 Tarun, S.Z. *et al.* (1997) Translation initiation factor eIF4G mediates *in vitro* poly(A) tail-dependent translation. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9046–9051
19 Wakiyama, M. *et al.* (2000) Interaction of eIF4G with poly(A)-binding protein stimulates translation and is critical for *Xenopus* oocyte maturation. *Curr. Biol.* 10, 1147–1150
20 Gray, N.K. *et al.* (2000) Multiple portions of poly(A)-binding protein stimulate translation *in vivo*. *EMBO J.* 19, 4723–4733
21 Le, H. *et al.* (1997) Translation initiation factors eIF-iso4G and eIF-4B interact with the poly(A)-binding protein and increase its RNA binding activity. *J. Biol. Chem.* 272, 16247–16255
22 Le, H. *et al.* (2000) The phosphorylation state of poly(A)-binding

protein specifies its binding to poly(A) RNA and its interaction with eukaryotic initiation factor (eIF) 4F, eIFiso4F, and eIF4B. *J. Biol. Chem.* 275, 17452–17462

23 Wei, C-C. *et al.* (1998) Wheat germ poly(A) binding protein enhances the binding affinity of eukaryotic initiation factor 4F and (iso)4F for cap analogue. *Biochemistry* 37, 1910–1916

24 von Der Haar, T. *et al.* (2000) Stabilization of eukaryotic initiation factor 4E binding to the mRNA 5′-Cap by domains of eIF4G. *J. Biol. Chem.* 275, 30551–30555

25 Borman, A.M. *et al.* (2000) Biochemical characterization of cap-poly(A) synergy in rabbit reticulocyte lysates: the eIF4G-PABP interaction increases the functional affinity of eIF4E for the capped mRNA 5′-end. *Nucleic Acids Res.* 28, 4068–4075

26 Searfoss, A. *et al.* (2001) Linking the 3′ poly(A) tail to the subunit joining step of translation initiation: relations of Pab1p, eukaryotic translation initiation factor 5b (Fun12p), and Ski2p-Slh1p. *Mol. Cell. Biol.* 21, 4900–4908

27 Craig, A.W. *et al.* (1998) Interaction of polyadenylate-binding protein with the eIF4G homologue PAIP enhances translation. *Nature* 392, 520–523

28 Grosset, C. *et al.* (2000) A mechanism for translationally coupled mRNA turnover: interaction between the poly(A) tail and a c-fos RNA coding determinant via a protein complex. *Cell* 103, 29–40

29 Bi, X. and Goss, D.J. (2000) Wheat germ poly(A)-binding protein increases the ATPase and the RNA helicase activity of translation initiation factors eIF4AeIF4B, and eIF-iso4F. *J. Biol. Chem.* 275, 17740–17746

30 Bushell, M. *et al.* (2001) Disruption of the interaction of mammalian protein synthesis eukaryotic initiation factor 4B with the poly(A)-binding protein by caspase- and viral protease-mediated cleavages. *J. Biol. Chem.* 276, 23922–23928

31 Hoshino, S. *et al.* (1999) The eukaryotic polypeptide chain releasing factor (eRF3/GSPT) carrying the translation termination signal to the 3′-Poly(A) tail of mRNA. Direct association of erf3/GSPT with polyadenylate-binding protein. *J. Biol. Chem.* 274, 16677–16680

32 Uchida, N. *et al.* (2002) A novel role of the mammalian GSPT/eRF3 associating with poly(A)-binding protein in Cap/Poly(A)-dependent translation. *J. Biol. Chem.* 277, 50286–50292

33 Mendez, R. and Richter, J.D. (2001) Translational control by CPEB: a means to the end. *Nat. Rev. Mol. Cell Biol.* 2, 521–529

34 de Moor, C.H. and Richter, J.D. (1999) Cytoplasmic polyadenylation elements mediate masking and unmasking of cyclin B1 mRNA. *EMBO J.* 18, 2294–2303

35 Minshall, N. *et al.* (1999) Dual roles of p82, the clam CPEB homologue, in cytoplasmic polyadenylation and translational masking. *RNA* 5, 27–38

36 Gebauer, F. and Richter, J.D. (1996) Mouse cytoplasmic polyadenylation element binding protein: an evolutionarily conserved protein that interacts with the cytoplasmic polyadenylation elements of c-mos mRNA. *Proc. Natl. Acad. Sci. U. S. A.* 93, 14602–14607

37 Stutz, A. *et al.* (1998) Masking, unmasking, and regulated polyadenylation cooperate in the translational control of a dormant mRNA in mouse oocytes. *Genes Dev.* 12, 2535–2548

38 Stebbins-Boaz, B. *et al.* (1999) Maskin is a CPEB-associated factor that transiently interacts with eIF-4E. *Mol. Cell* 4, 1017–1027

39 Gingras, A-C. *et al.* (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.* 68, 913–963

40 Cao, Q. and Richter, J.D. (2002) Dissolution of the maskin-eIF4E complex by cytoplasmic polyadenylation and poly(A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation. *EMBO J.* 21, 3852–3862

41 Hodgeman, R. *et al.* (2001) CPEB phosphorylation and cytoplasmic polyadenylation are catalyzed by the kinase IAK1/Eg2 in maturing mouse oocytes. *Development* 128, 2815–2822

42 Groisman, I. *et al.* (2000) CPEB, maskin, and cyclin B1 mRNA at the mitotic apparatus: implications for local translational control of cell division. *Cell* 103, 435–447

43 Stebbins-Boaz, B. *et al.* (1996) CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte maturation in *Xenopus*. *EMBO J.* 15, 2582–2592

44 Barkoff, A.F. *et al.* (2000) Translational control of cyclin B1 mRNA during meiotic maturation: coordinated repression and cytoplasmic polyadenylation. *Dev. Biol.* 220, 97–109

45 Wickens, M. *et al.* (2002) A PUF family portrait: 3′UTR regulation as a way of life. *Trends Genet.* 18, 150–157

46 Luitjens, C. *et al.* (2000) CPEB proteins control two key steps in spermatogenesis in *C. elegans*. *Genes Dev.* 14, 2596–2609

47 Ostareck, D.H. *et al.* (1997) mRNA silencing in erythroid differentiation: hnRNP K and hnRNP E1 regulate 15-lipoxygenase translation from the 3′ end. *Cell* 89, 597–606

48 Ostareck-Lederer, A. *et al.* (2002) c-Src-mediated phosphorylation of hnRNP K drives translational activation of specifically silenced mRNAs. *Mol. Cell. Biol.* 22, 4535–4543

49 Ostareck, D.H. *et al.* (2001) Lipoxygenase mRNA silencing in erythroid differentiation: the 3′UTR regulatory complex controls 60S ribosomal subunit joining. *Cell* 104, 281–290

50 Nakamura, A. *et al.* (2001) Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during *Drosophila* oogenesis. *Development* 128, 3233–3242

51 Johnstone, O. and Lasko, P. (2001) Translational regulation and RNA localization in *Drosophila* oocytes and embryos. *Annu. Rev. Genet.* 35, 365–406

52 Lie, Y.S. and Macdonald, P.M. (1999) Translational regulation of oskar mRNA occurs independent of the cap and poly(A) tail in *Drosophila* ovarian extracts. *Development* 126, 4989–4996

53 Castagnetti, S. *et al.* (2000) Control of oskar mRNA translation by Bruno in a novel cell-free system from *Drosophila* ovaries. *Development* 127, 1063–1068

54 Chuang, R.Y. *et al.* (1997) Requirement of the DEAD-Box protein ded1p for messenger RNA translation. *Science* 275, 1468–1471

55 Lasko, P. (2000) The *Drosophila melanogaster* genome: translation factors and RNA binding proteins. *J. Cell Biol.* 150, F51–F56

56 Carrera, P. *et al.* (2000) VASA mediates translation through interaction with a *Drosophila* yIF2 homolog. *Mol. Cell* 5, 181–187

57 Pestova, T.V. *et al.* (2000) The joining of ribosomal subunits in eukaryotes requires eIF5B. *Nature* 403, 332–335

58 Harris, A.N. and Macdonald, P.M. (2001) Aubergine encodes a *Drosophila* polar granule component required for pole cell formation and related to eIF2C. *Development* 128, 2823–2832

59 Chang, J.S. *et al.* (1999) The *Drosophila* CPEB homolog, orb, is required for oskar protein expression in oocytes. *Dev. Biol.* 215, 91–106

60 Wilhelm, J.E. *et al.* (2000) Isolation of a ribonucleoprotein complex involved in mRNA localization in *Drosophila* oocytes. *J. Cell Biol.* 148, 427–440

61 Mansfield, J.H. *et al.* (2002) Ypsilon Schachtel, a *Drosophila* Y-box protein, acts antagonistically to Orb in the oskar mRNA localization and translation pathway. *Development* 129, 197–209

62 Lau, N.C. *et al.* (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294, 858–862

63 Lee, R.C. and Ambros, V. (2001) An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* 294, 862–864

64 Lagos-Quintana, M. *et al.* (2001) Identification of novel genes coding for small expressed RNAs. *Science* 294, 853–857

65 Grosshans, H. and Slack, F.J. (2002) Micro-RNAs: small is plentiful. *J. Cell Biol.* 156, 17–21

66 Zeng, Y. *et al.* (2002) Both natural and designed micro RNAs can inhibit the expression of cognate mRNAs when expressed in human cells. *Mol. Cell* 9, 1327–1333

67 Lai, E.C. (2002) Micro RNAs are complementary to 3′ UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat. Genet.* 30, 363–364

68 Lai, E.C. and Posakony, J.W. (1997) The Bearded box, a novel 3′ UTR sequence motif, mediates negative post-transcriptional regulation of Bearded and Enhancer of split Complex gene expression. *Development* 124, 4847–4856

69 Lai, E.C. *et al.* (1998) The K box, a conserved 3′ UTR sequence motif, negatively regulates accumulation of enhancer of split complex transcripts. *Development* 125, 4077–4088

70 Palacios, I.M. and Johnston, D.S. (2001) Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu. Rev. Cell Dev. Biol.* 17, 569–614

71 Riechmann, V. and Ephrussi, A. (2001) Axis formation during *Drosophila* oogenesis. *Curr. Opin. Genet. Dev.* 11, 374–383

72 van Eeden, F.J. *et al.* (2001) Barentsz is essential for the posterior localization of oskar mRNA and colocalizes with it to the posterior pole. *J. Cell Biol.* 154, 511–523