Translational control in cellular and developmental processes

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Abstract | Growing evidence indicates that translational control of specific mRNAs contributes importantly to genetic regulation across the breadth of cellular and developmental processes. Synthesis of protein from a specific mRNA can be controlled by RNA-binding proteins at the level of translational initiation and elongation, and translational control is also sometimes coupled to mRNA localization mechanisms. Recent discoveries from invertebrate and vertebrate systems have uncovered novel modes of translational regulation, have provided new insights into how specific regulators target the general translational machinery and have identified several new links between translational control and human disease.

5′ cap structure
A 7-methylguanosine residue that is enzymatically added to the 5′ end of an mRNA and linked through a 5′–5′ triphosphate bridge.

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Translational control contributes immensely to the establishment of the intricate complexity of genetic regulation that is necessary for the development of multicellular organisms. It provides possibilities for controlling the spatial deployment of a protein that cannot be achieved through controlling transcription alone. Many translationally regulated mRNAs encode proteins whose correct distributions are essential for developmental processes, such as embryonic patterning, or for cellular processes, such as synaptic transmission[1]. Translational regulation of pre-existing mRNA can also provide a highly dynamic temporal response, which is exemplified by the immediate commencement after fertilization of global protein synthesis from maternally expressed and silenced mRNAs in the embryos of many species. The central role of post-transcriptional mechanisms of genetic regulation, including that of translational control, in establishing the proteome and enabling cellular and developmental processes is exemplified by the observation that only 40% of variability of protein levels in mouse embryonic fibroblasts is attributable to mRNA levels[2]. In addition, localization of mRNAs to particular subcellular regions is now known to be widespread[3] even in cells that are not as specialized as oocytes or neurons, where it has been most thoroughly studied. Localization of mRNA is usually coupled to translational control to restrict the distribution of the corresponding protein spatially.

This Review will explore various means by which interactions between RNA-binding proteins, translation initiation factors and the ribosome can be regulated in cellular and developmental processes. Regulation by microRNAs (miRNAs) is another extremely important mechanism of regulating translational activity and stability of mRNAs that has also been linked to genetic disease. However, this area has been extensively reviewed by others (for example, REFS 4,5) and will not be further considered here. Furthermore, translation of many viral and some cellular mRNAs is initiated through an alternative mechanism that is independent of the 5′ cap structure, in which the ribosome is recruited to a structured site on the mRNA called the internal ribosome entry site (IRES). IRES-mediated translation has been reviewed elsewhere[6,7] and will not be discussed in detail in this Review.

In this Review, we first discuss mechanisms that regulate the initiation of translation by modulating the binding of essential translation initiation factors to the 5′ cap structure. Next, we review processes that regulate translation by acting on the length of the poly(A) tail of target mRNAs. In subsequent sections, we turn to the regulation of mRNAs that have short open reading frames (ORFs) upstream of their main ORFs (these are known as upstream ORFs (uORFs)) and then to translational control at later stages of the process, such as ribosomal subunit joining and elongation. Processes by which ribosomal proteins function outside ribosomes to regulate translation is discussed in the next section, followed by a review of how post-transcriptional modifications of nucleotides in ribosomal RNAs (rRNAs) modulate ribosome function. Later sections of the Review include a discussion of translational masking, the targeting of mRNAs into translationally silent particles and how the phenomenon of mRNA localization is coupled to

translational regulation. Finally, examples are given that demonstrate how these processes are related to human disease.

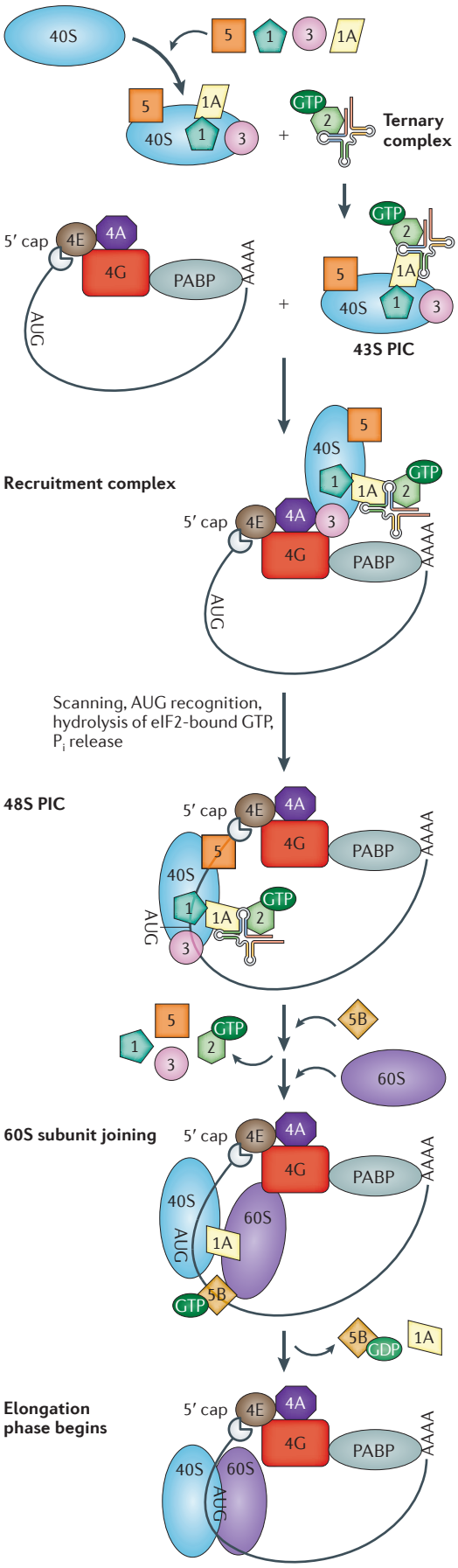
Translational control involving the 5′ cap structure

Translation proceeds through three phases — initiation, elongation and termination — and the initiation phase (FIG. 1) is subject to the greatest degree of regulation[8,9]. Most eukaryotic mRNAs are recruited to the ribosome through a mechanism that involves the 5′ 7-methylguanosine (^{7}mG) cap structure, to which initiator factors eventually bind to recruit the ribosome (FIG. 1). In this section, we consider how regulation of cap-dependent initiation is implicated in cell proliferation and development.

Translational control by eIF4E-binding proteins. Proteins that bind to the translation initiation factor eukaryotic translation initiation factor 4E (eIF4E), which are called eIF4E-binding proteins (4EBPs), can prevent ribosome recruitment by competing with the initiation factor eIF4G for binding to eIF4E. 4EBPs thereby block the assembly of eIF4F, which is itself a complex of eIF4E and two other initiation factors called eIF4A and eIF4G, and thereby repress translation (FIG. 2a,b). The binding affinity of 4EBPs to eIF4E is regulated by phosphorylation; increased phosphorylation promotes dissociation of 4EBPs and thus increases translational activity. The binding specificity of 4EBPs is regulated through an associated RNA-binding protein, which is often (but not always) a member of the PUF family (FIG. 2). How widespread this mechanism of translational regulation is can be seen in *Saccharomyces cerevisiae*, in which translation of over 1,000 mRNAs is regulated by either of two 4EBPs, Eap1 and Caf20, which associate with different PUF proteins[10].

Regulation by 4EBPs is important in cell cycle control. Activity of 4EBPs is modulated by intercellular signalling pathways that act on protein kinases. For example, 4EBP1 is a key effector of signalling pathways that regulate cell proliferation (and, in lower eukaryotes, cell size) in response to growth factors and nutrient status. Growth factors such as insulin promote the binding of 4EBP1 to the Raptor subunit of the mammalian target of rapamycin complex 1 (mTORC1), which is a key sensor of nutrient status[11,12] (FIG. 3). Activity of mTORC1 has been linked to phosphorylation of 4EBPs (in addition to phosphorylation of many other targets) and to consequent translational activation of a set of cell cycle regulators that drive cell cycle progression in mammalian cultured cells[13] (FIG. 3). Presumably related to this role, in response to nutrient status, mice that lack 4EBP1 and 4EBP2 are obese and have increased insulin resistance and increased fat accumulation[14]. These mice also show defects in myelopoiesis with increased titres of immature granulolytic precursor cells and a concomitant decrease in more mature cell types[15].

In *Drosophila melanogaster*, *Thor* (also known as *4EBP*) transcription can also be stimulated through TORC1. Transcription of *4EBP* is activated by the transcription factor forkhead box protein O (FOXO), which



Mammalian target of rapamycin complex 1 (mTORC1). A signalling complex that senses nutrient abundance and regulates cell growth and proliferation accordingly.

◀ Figure 1 | **An overview of cap-dependent translation initiation.** The small ribosomal subunit (40S) becomes associated with eukaryotic translation initiation factors (eIFs) 1, 1A, 3 and 5 (labelled as such in the figure) and then with the ternary complex, which is itself composed of the initiator Met-tRNA$_i$, eIF2 ('2') and GTP. This produces the 43S pre-initiation complex (PIC). Next, the mRNA is recruited to the 43S PIC through an association between the eIF4G subunit of the cap-binding complex and the 40S-bound eIF3 to form the recruitment complex. An association between eIF4G and poly(A)-binding protein (PABP) also promotes mRNA recruitment to the ribosome. After the mRNA is recruited, 40S that is bound to eIFs scans from 5′ to 3′ until an initiation codon is found; the complex is now termed the 48S initiation complex. Next, the large ribosomal subunit (60S) is recruited to the complex. This involves eIF5B ('5B') and the release of eIF2–GTP, eIF1, eIF3 and eIF5. After subunit joining, eIF1A and eIF5B–GDP are released. This marks the end of the initiation phase, and elongation then commences. For simplicity in all panels, some details and auxiliary factors have been omitted, and many multi-subunit components are represented by a single shape.

is positively regulated by the insulin-signalling pathway by AKT-dependent phosphorylation (FIG. 3). Increased FOXO and 4EBP activity enhances autophagy and removal of protein aggregates that accumulate over time in muscle tissue, thus regulating tissue ageing[16]. Related to this function of 4EBP in regulating tissue ageing, *D. melanogaster* 4EBP can extend lifespan under dietary restriction. 4EBP is activated under limited nutrients, leading to the preferential translation of a set of mRNAs that have less structured 5′ untranslated regions (UTRs). This set includes mRNAs that encode mitochondrial electron transport chain complexes, resulting in increased mitochondrial activity[17].

Acting through a different signalling pathway, other 4EBPs have been implicated in regulating cell proliferation in early embryonic development in *D. melanogaster* and *Xenopus laevis*. Maskin is a 4EBP that regulates translation of target mRNAs, including cyclin B1 (*ccnb1*), that contain cytoplasmic polyadenylation elements (CPEs) in their 3′UTRs[18]. Mitotic entry requires ccnb1, which is expressed during the G2 and M phases of the cell cycle but is destroyed and translationally silenced during G1 and S phases[19]. For *ccnb1* translation to occur, maskin has to dissociate from eIF4e to allow the eif4e–eif4g interaction. CPE-binding protein 1a (cpeb1a; see below) triggers the elongation of the *ccnb1* mRNA poly(A) tail, which enables binding of poly(A)-binding protein 1a (pabp1a), which also binds eif4g and promotes maskin dissociation from eif4e[20]. In *X. laevis* embryo extracts, the affinity of maskin for eif4e is primarily modulated by cdk1-dependent phosphorylation and calcineurin-dependent dephosphorylation, resulting in oscillating translation of *ccnb1* throughout the cell cycle[20,21]. Although characterized in early embryogenesis, 4EBPs such as maskin are likely to have a more widespread developmental role.

Another example of a 4EBP regulating translation in early development is the *D. melanogaster* 4EBP called CUP. In this case, the regulation is related to patterning rather than to cell cycle and proliferation, showing that this mode of translational control is involved in diverse processes. CUP represses the translation of *oskar* (*osk*) and *nanos* (*nos*) mRNAs, restricting these proteins to the posterior cytoplasm, which is essential for correct embryonic patterning (BOX 1,2). CUP is recruited to its target mRNAs by associating with particular RNA-binding proteins: Bruno (BRU) in the case of *osk*[22] and Smaug (SMG) in the case of *nos*[23]. However, CUP is much more functionally complex than mammalian 4EBPs. Although CUP indeed induces translational repression, surprisingly, this does not require its eIF4E-binding activity and thus does not involve competition for eIF4G[24,25]. Rather, CUP affects poly(A) tail length by recruiting the CCR4 deadenylase complex (see below) to its target mRNAs. CUP-associated mRNAs are not subsequently degraded, however, as they are protected by an amino-terminal regulatory domain of CUP through a mechanism that prevents decapping and requires one of the two eIF4E-binding motifs of CUP.

4EBPs may be involved in synaptic plasticity by their association with the fragile X mental retardation protein (FMRP), which is a neuronal RNA-binding protein that negatively regulates protein translation. Synaptic plasticity is necessary for learning and long-term memory and requires new protein synthesis, including local protein translation in dendrites and at the synapse[26]. FMRP can be recruited to eIF4E by a 4EBP called cytoplasmic FMR1-interacting protein 1 (CYFIP1). Reduction of CYFIP1 in neurons increased the levels of proteins encoded by mRNAs targeted by FMRP, such as *Map1b*, *aCamk2* and *App*[27], indicating a role for FMRP and CYFIP1 in regulating translation initiation. FMRP has also been implicated in regulation of translational elongation (see below).

Repression by competitive inhibition of eIF4E binding to the cap. Another mode of translational repression at the cap structure involves eIF4E homologous protein (4EHP). 4EHP is an eIF4E-related protein that binds to the 5′ cap structure but cannot bind eIF4G or 4EBPs, thus it represses translation by blocking assembly of eIF4F at the cap[28]. In *D. melanogaster*, translational control by 4EHP is involved in generating protein gradients that are essential for specifying embryonic pattern (BOX 1). 4EHP is recruited to *caudal* (*cad*) mRNA by bicoid (BCD) (FIG. 2b). *cad* encodes a transcriptional regulator that is involved in establishing anterior-to-posterior pattern in the early embryo. BCD binds to both 4EHP and the *cad* 3′UTR. As BCD is distributed in a gradient with the highest concentration in the anterior of the embryo, a posterior-to-anterior gradient of CAD is thus produced[29]. Another target of 4EHP-mediated translational control in *D. melanogaster* is *hunchback*. Hunchback is also a transcriptional regulator involved in anterior-to-posterior patterning. Recruitment of 4EHP to *hunchback* involves several proteins, including brain tumour (BRAT), nanos (NOS) and pumilio (PUM). In mammals, there is also evidence for 4EHP-dependent translational control of important developmental transcriptional regulators; homeobox B4 (*HOXB4*) mRNA translation is repressed by 4EHP[30,31], and HOXB4 is a transcriptional regulator implicated in stem cell self-renewal[32]. Cap-binding activity of human 4EHP, as well as its ability to inhibit translation of a reporter mRNA with the *cad* 3′UTR, is substantially increased by modification by the ubiquitin-like protein ISG15 (REF. 33). This

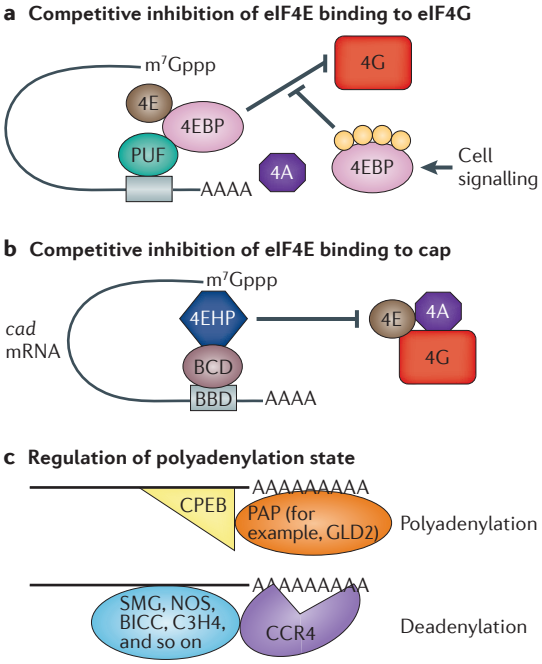


Figure 2 | **Three mechanisms for regulating translation of specific mRNAs.** In cap-dependent translation, recruitment of the mRNA to the small ribosomal subunit requires the association of eukaryotic translation initiation factor 4F (eIF4F) with the 5′ cap structure on the mRNA. eIF4F has three subunits called eIF4E, eIF4G and eIF4A (labelled '4E', '4G' and '4A' in the figure, repectively). The eIF4E subunit binds the cap structure. Mechanisms that inhibit eIF4E binding to the cap or that inhibit the assembly of eIF4F will in turn inhibit translation initiation. **a** | Translational repression by competitive inhibition of eIF4E binding to eIF4G. In this mechanism, an eIF4E-binding protein (4EBP) is recruited to a target mRNA by an RNA-binding protein, often of the PUF family. eIF4E binds to the 4EBP and is thus locally sequestered away from eIF4G, preventing cap-binding-complex assembly and thus recruitment of the mRNA to the 43S pre-initiation complex (PIC). Some types of 4EBP-mediated regulation are reversible by phosphorylation of the 4EBP (shown by the yellow circles), which in turn can be responsive to intercellular signalling mechanisms. **b** | Translational repression by competitive inhibition of eIF4E binding to the cap by an alternative cap-binding protein (namely, eIF4E2; also known as 4EHP) that cannot bind eIF4G. 4EHP is recruited to the target mRNA by an RNA-binding protein (bicoid (BCD) in the example illustrated), which prevents the recruitment of eIF4E and the assembly of a cap-binding complex. It is unknown whether and how this type of repression can be reversed. **c** | Translational regulation through effects on polyadenylation. Deadenylases such as CCR4 reduce translational activity, whereas poly(A) polymerases (PAPs) such as GLD2 increase translational activity. Deadenylases or polyadenylases can be recruited to target mRNAs by RNA-binding proteins that recognize specific sequence or structural elements. For simplicity, proteins that mediate the interactions between the RNA-binding protein and CCR4 or PAPs have been omitted from the drawing, and the multi-subunit CCR4 complex is represented as a single shape. BBD, Bicoid binding domain; BICC, bicaudal C; *cad*, *caudal*; CPEB, cytoplasmic polyadenylation element (CPE)-binding protein; NOS, nanos; SMG, Smaug.

indicates that 4EHP activity can be modulated by post-translational modifications. It is unknown how widespread 4EHP-type regulation is and whether it is linked to other developmental processes, but 4EHP orthologues have also been identified in *Arabidopsis thaliana* and *Caenorhabditis elegans*[28]. In addition, *D. melanogaster* encodes a second eIF4E-related protein, eIF4E6, that does not bind eIF4G and thus could function similarly to 4EHP[34].

Translational control and the poly(A) tail

In addition to control at the cap structure, translational control frequently involves effects on the poly(A) tails of target mRNAs. In general, lengthening the poly(A) tail promotes translational activity, whereas deadenylation leads to repression of translation and often to decapping and degradation of the mRNA. Many mRNAs that are important in germ cell development, neuronal synaptic plasticity and cellular senescence are translationally regulated through processes that affect their poly(A) tail length, which may be especially dynamic and finely tunable. This mode of regulation can be considered in terms of the competing activities of deadenylases and polymerases.

The multi-subunit CCR4 deadenylase complex is a major effector of this type of regulation. In the simplest case, a sequence-specific RNA-binding protein recruits the CCR4 complex to a specific mRNA, leading to a reduction in poly(A) tail length of that mRNA (FIG. 2c). Examples of proteins in *D. melanogaster* that recruit the CCR4 complex include: SMG, which mediates the degradation of maternal mRNAs at the maternal-to-zygotic transition[35]; Bicaudal C (BICC), which mediates autoregulation of *Bicc*[36]; and NOS, which regulates *cyclin B* mRNA[37]. In *X. laevis*, the zinc finger protein c3h4 recruits the ccr4 complex to target mRNAs that contain A/U-rich elements in their 3′UTRs, including F box protein 5 (*fbxo5a*; also known as *emi1*) and *fbxo43* (also known as *emi2*), the products of which are required for meiotic progression beyond metaphase I[38]. Links are also emerging between CCR4 and regulation mediated by small RNAs. For example, PIWI-associated RNAs (piRNAs) that are produced by transposable elements participate in recruiting SMG and CCR4 to the *nos* 3′UTR[39].

Cytoplasmic poly(A) polymerases (PAPs) operate antagonistically to deadenylases, lengthening the poly(A) tails of their targets and thus promoting translation. CPEB1A recruits PAPs to mRNAs that contain a particular U-rich element in their 3′UTRs. cpeb1a has been extensively studied in *X. laevis* oocytes, which remain quiescent in the first meiotic prophase until they have been stimulated by steroid hormones to resume the cell cycle; cpeb1a is a key component of this response. In unstimulated oocytes, cpeb1a is hypophosphorylated and contributes to translational repression[40]. Progesterone triggers the phosphorylation of cpeb1a, which leads to it acting to promote polyadenylation of a class of mRNAs that are involved in meiotic resumption[41–43]. CPEB1A regulation of meiosis occurs in mammals as well, as mice that lack CPEB1A fail to produce

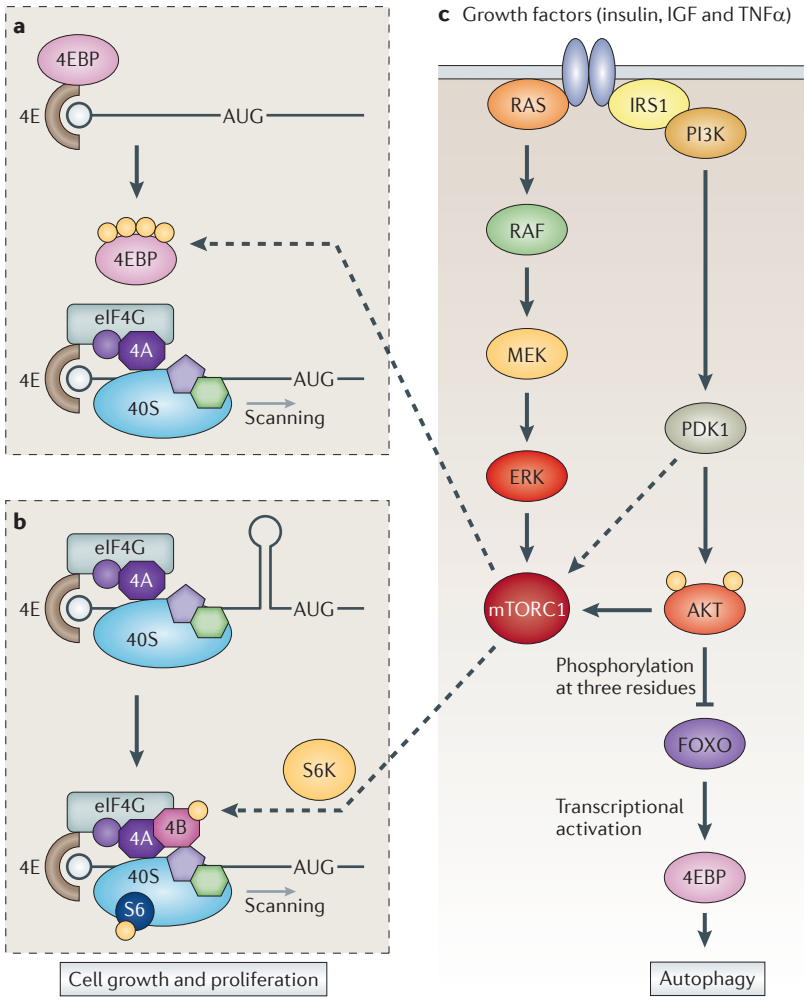


Figure 3 | **The mTORC1 pathway influences translation.** Evidence from invertebrate and vertebrate systems indicates that growth factors, such as insulin, insulin-like growth factor 1 (IGF1) or tumour necrosis factor-α (TNFα), stimulate cellular translation, cell survival and autophagy through the mammalian target of rapamycin complex 1 (mTORC1). Growth factors trigger mTORC1 signalling via the PI3K–AKT pathway or the RAS–RAF–MEK–ERK pathway. **a** | Activated mTORC1 phosphorylates multiple sites on the eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4EBP; shown by the yellow circles), which causes its dissociation from eIF4E (labelled '4E' in the figure), allowing binding of eIF4E to eIF4G ('4G'), assembly of an active cap-binding complex and recruitment of mRNA to the 40S ribosomal subunit. **b** | Activated mTORC1 promotes phosphorylation of eIF4B ('4B') and ribosomal protein S6 (RPS6; 'S6') by S6K. Phosphorylated eIF4B enhances the RNA helicase activity of eIF4A ('4A'), which is believed to promote the scanning step of translation initiation. **c** | The 4EBP is a downstream target of the transcription factor forkhead box protein O (FOXO), which is phosphorylated and thus inhibited by AKT. Under limited nutrients, the 4EBP promotes mitochondrial activity and autophagy. IRS1, insulin receptor substrate 1.

Pachytene
The stage during the first meiotic division when chromosomal crossing-over and recombination occurs.

Synaptonemal complex
A protein structure that forms between homologous chromosomes during the first meiotic prophase and that facilitates chromosome pairing.

viable germ cells and arrest meiosis at pachytene through effects on mRNAs encoding synaptonemal complex components[44].

In mammals, one of the targets of translational regulation by CPEB1A-mediated effects on the poly(A) tail is the tumour suppressor p53. p53 is a central regulatory molecule that triggers cell cycle arrest, senescence or apoptosis in response to cellular stress[45]. Expression of *TP53* (which encodes p53) is highly regulated at many different levels, including transcription, translation, control of mRNA and protein stability and post-translational modification of the protein. Translational control of an existing pool of *TP53* mRNA enables levels of the protein to increase rapidly in response to an activating stress signal.

Evidence for CPEB1A regulation of p53 came from human foreskin fibroblasts with knocked-down CPEB1A. These cells do not senesce and show reduced expression of p53 (REF. 46). In these cells, *TP53* mRNA had shorter poly(A) tails and was translated at lower levels. Further analysis revealed a complex mechanism. The vertebrate PAP GLD2 stabilizes *miR-122* by adding a single A residue to its 3′ end, and this miRNA represses CPEB1A expression[47]. Thus, GLD2 depletion results in *miR-122* depletion and increases CPEB1A expression. Another PAP, GLD4, is guided by CPEB1A to polyadenylate *TP53* mRNA and to promote its translation[48]. Therefore, reduction in GLD2 results in increased p53 expression[48]. Collectively, these examples illustrate that regulation of translation at the level of the poly(A) tail is likely to be widespread in development and in cellular processes (such as cell cycle versus senescence).

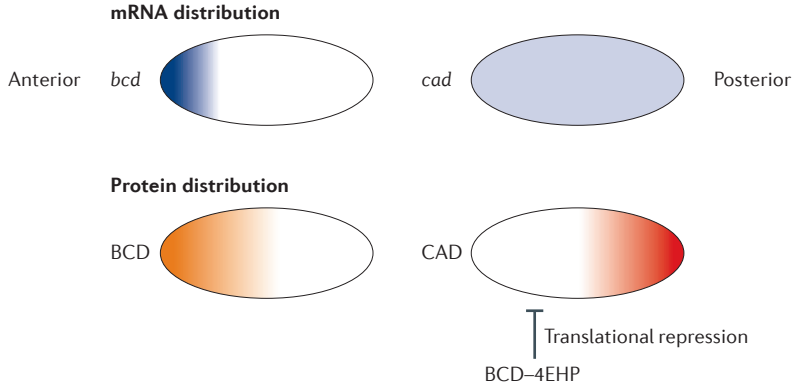
Negative regulation through uORFs

Some highly regulated mRNAs possess one or several uORFs[49]. Translating the main ORF thus requires resumption of ribosomal scanning, the efficiency of which depends on the length of the uORF, on the degree of secondary structure between the end of the uORF and the start of the main ORF and on proteins that may bind the mRNA between the uORF and the main ORF. A well-studied example is *male-specific lethal 2* (*msl2*) mRNA, which is regulated by Sex lethal (SXL) in *D. melanogaster*. In male flies, MSL2 is a part of a dosage compensation complex that is required for hypertranscription of the single X chromosome in males, which is necessary to equalize X-linked gene expression in males and females. Although *msl2* is required in males, its expression in females is lethal. Translation of *msl2* is repressed in females by the interaction of SXL with poly(U) tracts in both the 5′UTR and 3′UTR of *msl2* mRNA[50]. In the 3′UTR, SXL recruits the co-repressor Upstream of N-ras (UNR) to inhibit the association of the 43S pre-initiation complex to the 5′ end of the *msl2* mRNA[51]. SXL also binds to a poly(U) stretch downstream of the third of three uORFs in the 5′UTR; this promotes recognition of the translational start codon AUG of this uORF by the 43S pre-initiation complex, thus inhibiting further scanning and so repressing *msl2* translation[52]. As upstream translation initiation codons are found in around half of human and mouse transcripts and in nearly 60% of annotated *D. melanogaster* 5′UTRs, similar uORF-dependent regulatory mechanisms may be widespread[52].

Regulation at later stages of translation

Regulation of subunit joining. Translation may be regulated at stages later than the beginning of initiation; indeed, the subunit joining that marks the end of the initiation stage of translation is subject to regulation.

Box 1 | **Translational control in the *Drosophila melanogaster* early embryo**



At the beginning of embryogenesis, the single nucleus of the fertilized egg proceeds through a series of rapid mitotic divisions: doubling occurs every 9 minutes. During this time, transcription from the nuclei of the embryo is almost nonexistent; mRNAs and proteins that are expressed during oogenesis in germline cells called nurse cells, which are loaded into the egg, drive the early mitotic divisions and specify patterning[112]. Therefore, translational control is particularly important at this developmental stage. The nuclei that are generated by these rapid divisions migrate to the periphery of the embryo. Starting with the ninth division, the rate of mitosis begins to slow. Whereas the rest of the embryo remains syncytial, approximately five nuclei that have migrated to the posterior pole become enclosed by cell membranes to generate the primordial germ cells, called pole cells, and their divisions become asynchronous from those of the somatic nuclei, which pass through another four mitotic cycles without cytoplasmic division. At this stage, the embryo is called a syncytial blastoderm, and large-scale zygotic transcription begins.

In the early *Drosophila melanogaster* embryo, translational regulation of maternal mRNAs establishes anterior-to-posterior pattern, and this is usually coupled with mRNA localization. After localization, mRNAs are translated in the region where they are concentrated and are repressed elsewhere. For example, *bicoid* (*bcd*) is transcribed maternally in the nurse cells and is transferred into the oocyte during late oogenesis, and its mRNA becomes distributed in a steep anterior-to-posterior gradient, leading to an anterior-to-posterior distribution of BCD protein, which is a transcription factor[113]. Somatic nuclei at the periphery of the embryo experience different concentrations of BCD and activate different sets of downstream genes, depending on the concentration of BCD that they receive, thus resulting in further patterning. Translation of *caudal* (*cad*) is repressed by the recruitment of 4EBP by BCD, resulting in a posterior localization of CAD protein. Translation of *oskar* (*osk*) and *nanos* (*nos*), which encode proteins that are essential for posterior patterning and germ cell development, is restricted to the posterior pole of the oocyte and syncytial embryo through coupled mRNA localization and translational control (this is discussed further in the main text and in BOX 2). 4EHP, eIF4E homologous protein.

This stage involves the recruitment of the 60S ribosomal subunit and requires the general translation factor eIF5B. Regulation of this stage can occur by targeting of transcripts to ribonucleoproteins (RNPs) from which the 60S subunit is excluded, as is the case for *r15-LOX* mRNA that is silenced in premature human erythroid cells[53]. Repression of *r15-LOX* in this manner is mediated by a complex of three proteins that bind a 3′UTR element: heterogeneous nuclear ribonucleoprotein K (hnRNPK), hnRNPE1 (also known as PCBP1) and dead box helicase 6 (DDX6). Conversely, in *D. melanogaster*, subunit joining can be promoted by VASA, which is an RNA-binding protein that is maternally required for germ cell specification and posterior patterning. VASA interacts with eIF5B and induces translation of particular germline mRNAs. A mutant form of VASA with greatly reduced eIF5B-binding activity fails to activate translation of its targets, suggesting that its role may be to alleviate translational repression at the step of subunit joining[54,55]. VASA orthologues exist throughout the animal kingdom, but their specific role in translational regulation has not been thoroughly studied outside *D. melanogaster*. It is interesting to speculate that repressing translation at the final step of initiation, when protein synthesis is poised to begin, allows an instant response to a developmental or extracellular signal.

Regulation of translational elongation. Some examples exist of translational regulation of specific mRNAs at post-initiation phases of translation. In addition to the role of FMRP in initiation (as described above), this protein has been implicated in the regulation of elongation because in the brain a substantial proportion of FMRP is associated with polyribosomes[56]. High-throughput sequencing coupled with crosslinking and immunoprecipitation (HITS–CLIP) of FMRP-associated mRNAs identified 842 FMRP target transcripts in the mouse brain, many of which are enriched in synapses and are involved with neuronal plasticity[57]. FMRP is usually associated with coding sequences, and FMRP-associated mRNAs are associated with polyribosomes. These and other observations indicate that FMRP represses translation of its targets during the elongation stage by stalling ribosomal translocation, providing a molecular basis for FMRP-mediated synaptic function. Stalling elongation might be a mechanism that is suited to achieving the precise and rapid control of protein synthesis needed at neuronal synapses.

Another example of regulation at the level of translation elongation is the yeast protein Stm1, which inhibits translation by stalling the 80S ribosome on a target mRNA, most likely by directly binding the ribosome and causing it to pause in the elongation process[58]. The affected mRNAs can either be targeted for decapping and degradation or maintained in a paused status that would enable immediate resumption of translation. Stm1 orthologues do not seem to exist in higher eukaryotes, and Stm1 activity has not yet been linked to any specific cellular process.

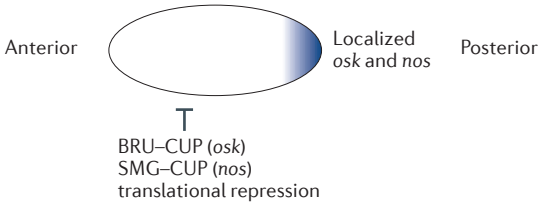
Regulation of elongation can also occur at the level of elongation factors. Phosphorylated eEF2 is a general inhibitor of translational elongation[59]. However, translation of certain neuronal mRNAs that are involved in memory processing actually increases under conditions of eEF2 hyperphosphorylation[60]. This is believed to occur because the reduction in general translational activity that is brought about by eEF2 hyperphosphorylation increases the availability of factors that promote translation initiation, thus weakening the effects of negative regulation that operates at this step. In *Aplysia californica* neurons, eEF2 phosphorylation can promote the translation of some mRNAs while repressing the translation of others, presumably through a similar mechanism, and the activity of eEF2 kinase can be differentially regulated at different positions within the cell[61].

eEF2 may also act as a platform for binding of regulatory proteins that block ribosome progression.

Ribonucleoproteins
Multimolecular complexes that contain both RNAs and proteins.

Heterogeneous nuclear ribonucleoprotein E1
(hnRNPE1). An RNA-binding protein with functions in precursor mRNA processing and in regulating mRNA stability and translation. Despite its name, it is present and functional both in nuclei and in the cytoplasm.

Box 2 | **CUP: an example of translational regulation in *Drosophila melanogaster* embryogenesis**



In *Drosophila melanogaster*, CUP is a eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4EBP) that is produced in nurse cells and is maternally deposited into the egg. CUP represses translation of *oskar* (*osk*) and *nanos* (*nos*) mRNAs — which encode important patterning proteins — before their posterior localization. This repression is mediated through interactions with the 3′ untranslated region (UTR)-binding proteins Bruno (BRU) and Smaug (SMG), which bind to *osk* and *nos*, respectively (see the figure). Although CUP was initially believed to repress translation by competing with eIF4G for eIF4E binding, recent work has shown that its function is more complex.

The amino-terminal regulatory domain of CUP contains two eIF4E-binding motifs: 4EBM1, which matches a sequence consensus that is common to many eIF4E-binding sites[114], and 4EBM2, which is non-canonical[23]. The carboxy-terminal region of CUP is glutamine-rich (Q-rich). The middle region and the Q-rich region, which are collectively referred to as the effector domain, associate with the CCR4 deadenylase complex and with decapping activators; thus, they have potent mRNA degradation activity[24]. However, the amino-terminal domain that includes 4EBM2 counteracts the activity of the effector domain through an unknown mechanism and protects the deadenylated and translationally repressed mRNA from further degradation by preventing it from being decapped. Repression of target mRNAs by CUP is complicated in that the effector domain can repress translation in the absence of deadenylation, and mRNAs that lack a cap structure can be repressed by CUP in cell-free extracts[25,115]. CUP binding to eIF4E appears to be primarily important not for repression but for sequestering eIF4E to the repressed mRNA to allow prompt translation activation after dissociation of CUP. CUP also modulates the phosphorylation of eIF4E to regulate its function[116]. As CUP is required for other processes in oogenesis, including cyst formation, chromosome condensation and oocyte development and growth, it must have additional mRNA targets beyond *osk* and *nos*[114].

This mechanism of translation regulation occurs on hypoxia-inducible factor 1 alpha (*HIF1A*) RNA, which rapidly increases in response to oxidative stress[62] and is translationally repressed under normoxia. Repression of *HIF1A* translation is accomplished by CPEB2, a protein that is related to CPEB. CPEB2 binds to the 3′UTR of *HIF1A* mRNA and to eEF2 and slows translation elongation[63]. Under hypoxic conditions, CPEB2 dissociates from *HIF1A* mRNA. The translation factor eEF1A1 can act in a similar manner during epithelial-to-mesenchymal transitions (EMTs), in which epithelial cells undergo a developmental switch to a highly motile mesenchymal state. EMTs occur in some normal developmental processes and also in tumours, where they drive metastasis. EMTs can be induced by transforming growth factor-β (TGFβ), which stimulates a kinase cascade that ultimately phosphorylates the RNA-binding protein hnRNPE1. Unphosphorylated hnRNPE1 binds to a 3′UTR element and inhibits translation of target mRNAs by binding to eEF1A1, blocking progression of the ribosome[64]. Phosphorylation of hnRNPE1 releases it from its binding site, thus activating translation.

Recently, it was demonstrated that in both *C. elegans* and human cells, PUF proteins can form an inhibitory complex with miRNA-binding proteins of the Argonaute (AGO) family and with eEF1A, which is another translation elongation factor[65]. Inhibition of translation appears to involve a negative effect on the GTPase activity of eEF1A by PUF and AGO, which is accompanied by a subsequent stronger repression that may affect release of the nascent polypeptide from the ribosome. Whether miRNAs are involved in this process — for instance, by targeting the inhibitory complex to particular mRNAs — remains to be investigated.

Regulation by components of the ribosome

In addition to their constitutive function in translation, some ribosomal proteins are actively involved in translational control of certain genes or sets of genes. Recent findings support specific and regulatory functions of ribosomes or ribosomal proteins in embryonic development, neuronal synaptic function and cell survival.

Ribosomal proteins as regulators of cell proliferation and cell survival. In cultured human and mouse cells, ribosomal protein L26 (RPL26) operating outside of ribosomes is required for full activation of *TP53* mRNA translation after DNA damage, redistributing it into heavier polysomes[66]. To exert its effects, RPL26 binds to a double-stranded RNA structure that can form from complementary sequences in the 5′ and 3′UTRs. Preventing formation of this structure abrogates RPL26-mediated activation[66,67]. Precisely how binding of RPL26 to the structure stimulates translation remains unclear, although it is known that this form of translation activation is regulated by the E3 ubiquitin ligase MDM2, which is a negative regulator of p53. Under non-stressed conditions, p53 protein is kept at a low level by the inhibitory binding of MDM2, resulting in its ubiquitylation[68]. MDM2 also specifically binds to RPL26, promoting its degradation and sequestering it from *TP53* mRNA. In response to genotoxic stress, RPL26 is released from MDM2 (REF. 69). Thus RPL26 is one of the important components of an MDM2–p53 autoregulatory feedback loop that controls cell cycle arrest and apoptosis.

Other ribosomal proteins are also thought to regulate the levels of p53. In mice, two mutations causing pigmentation defects affect ribosomal protein S19 (*Rps19*)

Polyribosomes
Actively translated mRNAs that are associated with multiple ribosomes, each elongating a different nascent polypeptide chain.

High-throughput sequencing coupled with crosslinking and immunoprecipitation
(HITS–CLIP). A technique in which mRNAs associated with a particular protein or in a ribonucleoprotein complex are recovered by co-immunoprecipitation and analysed by deep sequencing.

Normoxia
A physiological condition in which oxygen levels are sufficient and not limiting for metabolic processes.

MDM2
An E3 ubiquitin ligase that recognizes and destabilizes p53.

Box 3 | **Examples of links between translational control and human disease**

The importance of translational control in developmental and cellular processes is shown by links between translational control and human disease. Although space does not permit a fully comprehensive discussion of all instances in which perturbations in translational control have been connected to disease, the examples below illustrate how the pathways discussed in this Review have been linked to cancer, neurological disease and rare genetic disorders.

eIF4E and cancer

Consistent with the important role of the balance between eukaryotic translation initiation factor 4E (eIF4E)-binding proteins (4EBPs) and eIF4E in regulating of cell proliferation, there is extensive evidence linking misexpression or misregulation of these proteins to cancer. eIF4E is overexpressed in many cancers, ectopic expression of eIF4E is oncogenic, and hyperphosphorylated 4EBPs are adverse prognostic indicators. Recently, a small molecule was identified that inhibits interactions of eIF4E with eIF4G or 4EBP1. This molecule was also demonstrated to reverse chemoresistance in the mouse Eμ–MYC lymphoma model[117].

4EBPs and Parkinson's disease

4EBPs can be phosphorylated by leucine-rich repeat kinase 2 (LRRK2), and dominant mutations in *LRRK2* are frequently found in Parkinson's disease. 4EBPs are substrates for LRRK2, and in *Drosophila melanogaster*, *LRRK2* mutants affect dopaminergic survival, at least in part through changes in 4EBP activity[118]. Two additional genes, *PINK1* and *parkin*, that operate in a common pathway have been implicated in human Parkinson's disease, and mutations in the counterpart genes in *D. melanogaster* result in neurodegeneration and locomotory phenotypes that are related to Parkinson's disease[119]. Concomitant loss of 4EBPs drastically worsens these phenotypes, producing lethality, whereas overexpression of 4EBPs substantially suppresses them[120].

Fragile X syndrome and related disorders

Fragile X syndrome, which is the most common cause of intellectual disability in males, is caused by triplet expansions in the promoter region of the gene encoding fragile X mental retardation protein (FMRP) that reduce or eliminate its expression. There is also evidence linking cytoplasmic FMR1-interacting protein 1 (CYFIP1) and eIF4E to autism spectrum disorder and Prader–Willi syndrome[121].

Ribosomes and genetic disease

As mentioned in the text, mutations affecting genes that encode any of several ribosomal proteins are associated with Diamond–Blackfan anaemia. In addition, dyskeratosis congenita 1 (DKC1), which is the gene that encodes pseudouridine synthase, is mutated in X-linked dyskeratosis syndrome — a rare human disease with symptoms including bone marrow failure, skin abnormalities and an increased risk of cancer. Mutations in small nucleolar RNA genes have been associated with Prader–Willi syndrome[122].

and *Rps20* (REF. 70), which encode ribosomal proteins. These mutants, as well as mutations in *Rps6*, show increased levels of kit ligand (*Kitl*) mRNA and increased levels of p53, which is essential for the pigmentation phenotypes. *Rpl24*-mutant mice show some related phenotypes and also show upregulation of p53 (REF. 71). How these ribosomal proteins regulate p53 level remains to be elucidated. The mouse phenotypes are related to those of the human genetic disorder Diamond–Blackfan anaemia (DBA; BOX 3), which is linked to germline heterozygous mutations in the genes *Rps17*, *Rps19*, *Rps24*, *Rpl5*, *Rpl11* or *Rpl35a*[72–76] that encode ribosomal proteins. The fact that mutations in so many different ribosomal protein genes produce related phenotypes suggests that the effects observed result from ribosomal haploinsufficiency and a concomitant reduction in translational activity. Consistent with this, reduction of *Rps19* and *Rpl11* expression in p53-deficient erythroblast cells reduced polyribosome association and translation of a specific set of transcripts, including two genes — BCL2-associated athanogene (*Bag1*) and cold shock domain containing E1 (*Csde1*) — that encode essential proteins required for erythrocyte differentiation[77]. Probably related to this role in control of translation of p53, heterozygous mutations in ribosomal protein genes in zebrafish cause cancers in the embryo[78]. Furthermore, knockdown of *rps7* in zebrafish results in elevated p53 expression and p53-mediated cell cycle arrest and apoptosis, as well as in other developmental abnormalities that can be partially rescued by simultaneous knockdown of p53 (REF. 79).

Ribosomal proteins in developmental regulation. It is not only p53 that is translationally regulated by ribosomal proteins: they may also have a role in translational regulation during development. A dominant mutation in mice called tail short, which was first identified in 1950, and two other mutations with similar phenotypes all result from alterations in the ribosomal protein gene *Rpl38* (REF. 80). These mutations cause skeletal patterning abnormalities, including anterior-to-posterior and posterior-to-anterior homeotic transformations, eye abnormalities and numerous other developmental defects, such as cleft palate and exencephaly. Many of these phenotypes are consistent with alterations in Homeobox gene (HOX gene) expression. Although global translation was unaffected in the mutants, for 8 of 39 HOX genes, the level of 80S monosome-associated and polysome-associated HOX gene mRNA was substantially reduced by as much as nearly tenfold. Corresponding protein levels were also reduced, indicating a specific regulatory role for RPL38. Mutations in other ribosomal protein genes did not lead to similar effects. Quantitative expression profiling also revealed that not only RPL38 but also most ribosomal proteins show a great deal of temporal and tissue heterogeneity in their expression during embryogenesis, which suggests that they may also have other such specialized functions.

Finally, there has been intriguing recent evidence that translation may be regulated by modifications of ribosomal proteins. Many nucleotide bases of rRNAs are enzymatically modified, either by methylation or by conversion of uridine to pseudouridine. These modifications are evolutionarily conserved and are often clustered in functionally important parts of the ribosome. Ribosomes from yeast, mouse or human cells with reduced pseudouridine synthase activity bound less well both to charged tRNA and to a viral internal ribosome entry site (IRES), and they had decreased reading frame fidelity, indicating deficiencies in translation[81]. These results help to explain translational control defects observed in cells from mice with mutations in the pseudouridine synthase gene *DKC1* or from human patients with mutations in the homologous gene[82,83] (BOX 3). Enzymes that modify rRNA residues are guided to the correct sites by small nucleolar RNAs (snoRNAs). A recent study in zebrafish reported that snoRNA depletion results in extensive developmental defects and embryonic lethality[84].

Prader–Willi syndrome
A rare genetic disorder that results in obesity and reductions in muscle tone, cognitive capacity and production of sex hormones.

Homeotic transformation
In developmental biology, a situation that is often caused by a mutation or an alteration in gene expression whereby precursors to a particular cell, tissue or organ type develop instead into a different one.

Sequestering mRNAs into inactive particles

mRNAs are often components of ribonucleoprotein particles or larger complexes, and the nature of these complexes contributes to translational control. For example, it has been recognized for nearly half a century that in *X. laevis*, translationally repressed maternal mRNAs are packaged into large cytoplasmic bodies called processing bodies. Related structures called stress granules form under stress conditions, such as glucose starvation or hypoxia, and the term 'RNA granules' has been proposed to refer to all such particles collectively[85]. The molecular composition of various types of RNA granules and the means by which they assemble are beginning to be understood[86]. RNA granules in *D. melanogaster* embryos that contain translationally repressed *nos* include SMG, CUP, maternal expression at 31B (ME31B), trailer hitch (TRAL) and at least two subunits of the CCR4 complex[25]. ME31B and TRAL have both been implicated in translational repression and frequently associate with translationally silent RNP particles. The repressor complex excludes eIF4G and thus blocks 48S initiation complex formation on the associated *nos* mRNA. However, a later step in translation is also targeted as IRES-dependent translation and is also repressed in these RNP particles, suggesting that multiple mechanisms are at play.

Further insight into the nature of RNA granules came from a study of polypyrimidine tract binding protein (PTB) in *D. melanogaster*, which is required for translational repression of *osk* during early oogenesis[87]. PTB is found throughout evolution and has been linked to many nuclear and cytoplasmic processes involving RNA[88]. In the *D. melanogaster* oocyte, PTB binds with a high affinity and cooperativity to the *osk* 3′UTR at several pyrimidine-rich sites and catalyses oligomerization of multiple *osk* mRNA molecules through bridging interactions. A remaining question is whether proteins such as PTB can achieve coordinate regulation of more than one species of mRNA by nucleating assembly of mRNPs. However, there is some observational evidence to the contrary, as particular mRNA species are clustered into specific domains within particles[89]. PTB may act in other cell types, as several mRNAs expressed in rat pancreatic β-cells that encode protein components of insulin secretory granules are bound, stabilized and translationally upregulated by PTB[90].

mRNA localization and translational control

In addition to sequestration for repression, mRNA localization to a particular cytoplasmic compartment often contributes to the spatial restriction of the distribution of the corresponding protein. This process has been studied in many cell types, including embryos, oocytes, neurons and polarized epithelial cells[91]. Localization of mRNAs must be coupled to translational regulation to be maximally effective. Localized mRNA is translationally active when it is at a high concentration and translationally repressed elsewhere. Disruption of mRNA localization pathways can result in ectopic expression of proteins; for example, in the *D. melanogaster* female germline, this can result in a failure to complete oogenesis or a failure in embryonic patterning defects that cause lethality[92]. It is estimated that about 70% of mRNAs in early *D. melanogaster* embryos are localized, and over 50 mRNAs localize to mouse fibroblast pseudopodia[93]. In differentiated neurons, dendritically localized mRNAs allow rapid responses to neuronal stimulation, which has a key role in synaptic plasticity[94,95]. Therefore, this mechanism is likely to be widespread throughout development.

As discussed above, mRNA localization is important in embryonic patterning, and in *D. melanogaster* mRNA

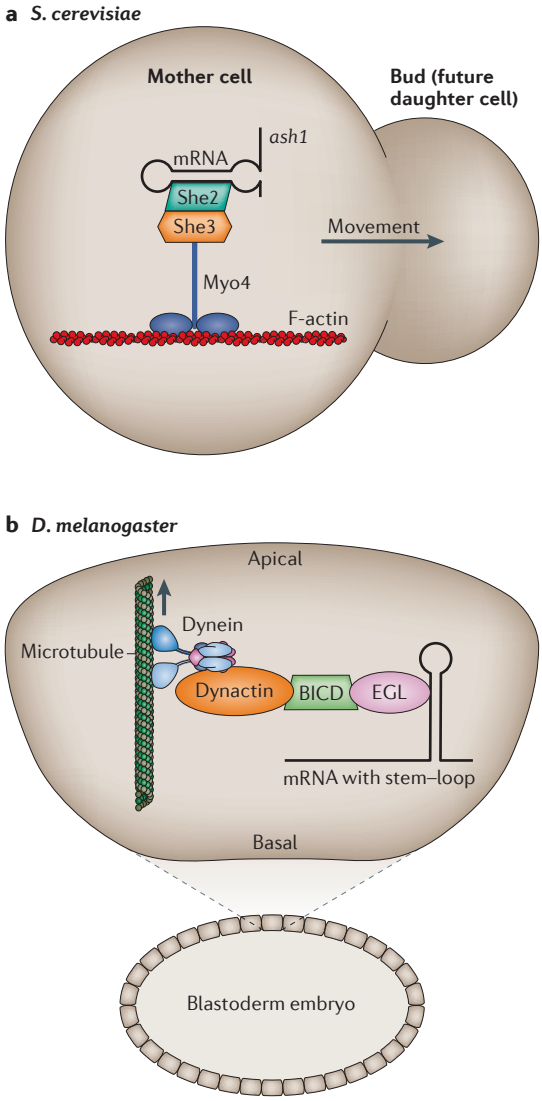


Figure 4 | **Linking mRNAs to motor proteins for localization.** **a** | Localization of *ash1* mRNA to the bud tip in dividing *Saccharomyces cerevisiae* cells involves myosin (Myo4)-driven transport along F-actin filaments. Two proteins, called She2 and She3, mediate the linkage; She2 binds to a stem–loop region of the *ash1* 3′ untranslated region. **b** | Apical localization of mRNAs in *Drosophila melanogaster* blastoderm cells involves dynein-driven transport along microtubules. mRNAs are linked through an interaction of the egalitarian–Bicaudal D (EGL–BICCD) complex to dynactin, which in turn binds to dynein.

Homeobox gene
(HOX gene). One of a set of genes that encodes a particular type of transcription factor and that is implicated in establishing many developmental fates, including the identity of body segments along the anterior–posterior axis.

Processing bodies
Also called P bodies, these constitute a type of RNA granule that is linked to cytoplasmic RNA decay pathways.

Ribosome profiling
A technique for measuring translation of many species of mRNA simultaneously *in vivo*.

transport and localization has been studied with a particular focus on processes that occur during oogenesis and early embryogenesis. In embryonic blastoderm cells, mRNAs are directed to the apical cytoplasm by a dynein-dependent minus-end-directed microtubule cargo transport mechanism. A complex of two proteins — egalitarian (EGL) and Bicaudal D (BICD) — is directly responsible for linking mRNAs to dynein and to microtubules[96,97] (FIG. 4a). EGL binds to a localization element in the mRNA to be transported and to BICD[97], which in turn interacts with dynein through dynactin[98,99]. Localization of *oskar* (*osk*) mRNA to the posterior of the *D. melanogaster* oocyte also proceeds through microtubule-dependent motor-driven transport, but the mechanism is more complex[100–105].

RNA localization can also be achieved by zipcodes, which are RNA sequence elements that are usually located in the 3′UTR and are binding sites for proteins that mediate localization. An example of the use of these zipcodes is the localization of β-actin mRNA to pseudopodia in chicken embryo fibroblasts. Here, transport along both actin microfilaments and microtubules is mediated through an association with zipcode-binding protein 1 (ZBP1), which is also involved in its translational regulation[106]. Localization mechanisms are also important in ensuring the correct distribution of proteins between daughter cells of *S. cerevisiae* following asymmetric division by budding[107–109] (FIG. 4b).

Conclusion

Translational control has an impact on many cellular and developmental processes, and most steps of translation are subject to specific regulation. New results in this area are rapidly emerging, and ribosome profiling, which is a new quantitative technique for precise genome-wide monitoring of translational activity[110], promises to revolutionize this field further when it becomes more widely used to study developing multicellular systems.

mRNAs can be translationally active, stored in translationally quiescent particles or targeted for degradation. How these options are regulated for a particular mRNA and how this regulation responds to different cellular conditions remain important open questions for most developmentally regulated mRNAs. Also of interest is emerging evidence that the same protein can influence several stages of post-transcriptional gene regulation, as exemplified above with CUP. Finally, whereas a substantial amount has been learned about processes that repress or silence translation, less is understood about how these mechanisms can be overcome in cellular or developmental contexts in which translation needs to be activated.

Much of what has been learned about translational control in development has been derived from forward genetics approaches in which a particular gene encoding a regulatory protein is mutated and consequent effects are measured. If the functional units in translational control are actually RNA–protein complexes and a given protein may be a component of several such complexes, then inactivating one gene might perturb multiple functional units. This might explain why so many mutations affecting translational control pathways give pleiotropic phenotypes. Molecular approaches will be necessary to unravel this; more efficient techniques are required to identify particular species of RNA–protein complexes from developing cells and tissues and to determine their compositions. Potential approaches could be based on quantitative imaging and on improved affinity-purification techniques, such as those successfully used to help elucidate RNP biogenesis pathways in yeast[111]. In addition, further comparisons of transcriptomes with proteomes in particular cell types and at particular stages of development will improve the definition of the scope of translational control.

Unlike transcriptional control, which is restricted to the nucleus, translational control mechanisms operate throughout the cell and can regulate expression of cytoplasmic proteins to ensure that they are present at the positions and times that they are required. Through the experimental approaches outlined above, as well as through complementary techniques, a better picture of the crucial role of translational control in cellular and developmental processes is certain to emerge, as is a more widespread appreciation of its enormous role in genetic regulation.

1. Loya, C. M., Van Vactor, D. & Fulga, T. A. Understanding neuronal connectivity through the post-transcriptional toolkit. *Genes Dev.* **24**, 625–635 (2010).
2. Schwanhausser, B. *et al.* Global quantification of mammalian gene expression control. *Nature* **473**, 337–342 (2011).
This is a comprehensive and careful study that documents the widespread nature of post-transcriptional genetic regulation.
3. Lecuyer, E. *et al.* Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* **131**, 174–187 (2007).
4. Esteller, M. Non-coding RNAs in human disease. *Nature Rev. Genet.* **12**, 861–874 (2011).
5. Krol, J., Loedige, I. & Filipowicz, W. The widespread regulation of microRNA biogenesis, function and decay. *Nature Rev. Genet.* **11**, 597–610 (2010).
6. Filbin, M. E. & Kieft, J. S. Toward a structural understanding of IRES RNA function. *Curr. Opin. Struct. Biol.* **19**, 267–276 (2009).
7. Shatsky, I. N., Dmitriev, S. E., Terenin, I. M. & Andreev, D. E. Cap- and IRES-independent scanning mechanism of translation initiation as an alternative to the concept of cellular IRESs. *Mol. Cells* **30**, 285–293 (2010).
8. Jackson, R. J., Hellen, C. U. & Pestova, T. V. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Rev. Mol. Cell Biol.* **11**, 113–127 (2010).
9. Sonenberg, N. & Hinnebusch, A. G. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* **136**, 731–745 (2009).
10. Cridge, A. G. *et al.* Identifying eIF4E-binding protein translationally-controlled transcripts reveals links to mRNAs bound by specific PUF proteins. *Nucleic Acids Res.* **38**, 8039–8050 (2010).
11. Hay, N. & Sonenberg, N. Upstream and downstream of mTOR. *Genes Dev.* **18**, 1926–1945 (2004).
12. Rapley, J., Oshiro, N., Ortiz-Vega, S. & Avruch, J. The mechanism of insulin-stimulated 4E-BP protein binding to mammalian target of rapamycin (mTOR) complex 1 and its contribution to mTOR complex 1 signaling. *J. Biol. Chem.* **286**, 38043–38053 (2011).
13. Dowling, R. J. *et al.* mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science* **328**, 1172–1176 (2010).
14. Le Bacquer, O. *et al.* Elevated sensitivity to diet-induced obesity and insulin resistance in mice lacking 4E-BP1 and 4E-BP2. *J. Clin. Invest.* **117**, 387–396 (2007).
15. Olson, K. E., Booth, G. C., Poulin, F., Sonenberg, N. & Beretta, L. Impaired myelopoiesis in mice lacking the repressors of translation initiation, 4E-BP1 and 4E-BP2. *Immunology* **128**, e376–e384 (2009).
16. Demontis, F. & Perrimon, N. FOXO/4E-BP signaling in *Drosophila* muscles regulates organism-wide proteostasis during aging. *Cell* **143**, 813–825 (2010).
This is a comprehensive study of muscle ageing that implicates 4EBPs in the removal of damaged and aggregated protein.
17. Zid, B. M. *et al.* 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*. *Cell* **139**, 149–160 (2009).

18. Cao, Q. & Richter, J. D. 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 (2002).
19. Groisman, I., Jung, M. Y., Sarkissian, M., Cao, Q. & Richter, J. D. Translational control of the embryonic cell cycle. *Cell* **109**, 473–483 (2002).
20. Barnard, D. C., Cao, Q. & Richter, J. D. Differential phosphorylation controls Maskin association with eukaryotic translation initiation factor 4E and localization on the mitotic apparatus. *Mol. Cell Biol.* **25**, 7605–7615 (2005).
21. Cao, Q., Kim, J. H. & Richter, J. D. CDK1 and calcineurin regulate Maskin association with eIF4E and translational control of cell cycle progression. *Nature Struct. Mol. Biol.* **13**, 1128–1134 (2006).
22. Nakamura, A., Sato, K. & Hanyu-Nakamura, K. *Drosophila* Cup is an eIF4E binding protein that associates with Bruno and regulates *oskar* mRNA translation in oogenesis. *Dev. Cell* **6**, 69–78 (2004).
23. Nelson, M. R., Leidal, A. M. & Smibert, C. A. *Drosophila* Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression. *EMBO J.* **23**, 150–159 (2004).
24. Igreja, C. & Izaurralde, E. CUP promotes deadenylation and inhibits decapping of mRNA targets. *Genes Dev.* **25**, 1955–1967 (2011).
25. Jeske, M., Moritz, B., Anders, A. & Wahle, E. Smaug assembles an ATP-dependent stable complex repressing *nanos* mRNA translation at multiple levels. *EMBO J.* **30**, 90–103 (2011).
26. Richter, J. D. & Klann, E. Making synaptic plasticity and memory last: mechanisms of translational regulation. *Genes Dev.* **23**, 1–11 (2009).
27. Napoli, I. *et al.* The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell* **134**, 1042–1054 (2008).
28. Rosettani, P., Knapp, S., Vismara, M. G., Rusconi, L. & Cameron, A. D. Structures of the human eIF4E homologous protein, h4EHP, in its m^{7}GTP-bound and unliganded forms. *J. Mol. Biol.* **368**, 691–705 (2007).
29. Cho, P. F. *et al.* A new paradigm for translational control: inhibition via 5′–3′ mRNA tethering by Bicoid and the eIF4E cognate 4EHP. *Cell* **121**, 411–423 (2005).
30. Cho, P. F. *et al.* Cap-dependent translational inhibition establishes two opposing morphogen gradients in *Drosophila* embryos. *Curr. Biol.* **16**, 2035–2041 (2006).
31. Villaescusa, J. C. *et al.* Cytoplasmic Prep1 interacts with 4EHP inhibiting Hoxb4 translation. *PLoS ONE* **4**, e5213 (2009).
32. Antonchuk, J., Sauvageau, G. & Humphries, R. K. HOXB4-induced expansion of adult hematopoietic stem cells *ex vivo*. *Cell* **109**, 39–45 (2002).
33. Okumura, F., Zou, W. & Zhang, D. E. ISG15 modification of the eIF4E cognate 4EHP enhances cap structure-binding activity of 4EHP. *Genes Dev.* **21**, 255–260 (2007).
34. Hernandez, G. *et al.* Functional analysis of seven genes encoding eight translation initiation factor 4E (eIF4E) isoforms in *Drosophila*. *Mech. Dev.* **122**, 529–543 (2005).
35. Tadros, W. *et al.* SMAUG is a major regulator of maternal mRNA destabilization in *Drosophila* and its translation is activated by the PAN GU kinase. *Dev. Cell* **12**, 143–155 (2007).
36. Chicoine, J. *et al.* Bicaudal-C recruits CCR4-NOT deadenylase to target mRNAs and regulates oogenesis, cytoskeletal organization, and its own expression. *Dev. Cell* **13**, 691–704 (2007).
37. Kadyrova, L. Y., Habara, Y., Lee, T. H. & Wharton, R. P. Translational control of maternal *Cyclin B* mRNA by Nanos in the *Drosophila* germline. *Development* **134**, 1519–1527 (2007).
38. Belloc, E. & Mendez, R. A deadenylation negative feedback mechanism governs meiotic metaphase arrest. *Nature* **452**, 1017–1021 (2008).
39. Rouget, C. *et al.* Maternal mRNA deadenylation and decay by the piRNA pathway in the early *Drosophila* embryo. *Nature* **467**, 1128–1132 (2010).
40. de Moor, C. H. & Richter, J. D. Cytoplasmic polyadenylation elements mediate masking and unmasking of *cyclin B1* mRNA. *EMBO J.* **18**, 2294–2303 (1999).
41. Charlesworth, A., Cox, L. L. & MacNicol, A. M. Cytoplasmic polyadenylation element (CPE)- and CPE-binding protein (CPEB)-independent mechanisms regulate early class maternal mRNA translational activation in *Xenopus* oocytes. *J. Biol. Chem.* **279**, 17650–17659 (2004).
42. Keady, B. T., Kuo, P., Martinez, S. E., Yuan, L. & Hake, L. E. MAPK interacts with XGef and is required for CPEB activation during meiosis in *Xenopus* oocytes. *J. Cell Sci.* **120**, 1093–1103 (2007).
43. Mendez, R. *et al.* Phosphorylation of CPE binding factor by Eg2 regulates translation of *c-mos* mRNA. *Nature* **404**, 302–307 (2000).
44. Tay, J. & Richter, J. D. Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. *Dev. Cell* **1**, 201–213 (2001).
45. Vilborg, A., Wilhelm, M. T. & Wiman, K. G. Regulation of tumor suppressor p53 at the RNA level. *J. Mol. Med.* **88**, 645–652 (2010).
46. Burns, D. M. & Richter, J. D. CPEB regulation of human cellular senescence, energy metabolism, and p53 mRNA translation. *Genes Dev.* **22**, 3449–3460 (2008).
47. Katoh, T. *et al.* Selective stabilization of mammalian microRNAs by 3′ adenylation mediated by the cytoplasmic poly(A) polymerase GLD-2. *Genes Dev.* **23**, 433–438 (2009).
48. Burns, D. M., D'Ambrogio, A., Nottrott, S. & Richter, J. D. CPEB and two poly(A) polymerases control *miR-122* stability and *p53* mRNA translation. *Nature* **473**, 105–108 (2011).
In this study, the authors demonstrate that a miRNA can be a target for cytoplasmic polyadenylation and explain why CPEB, which promotes polyadenylation, nevertheless negatively regulates *TP53* mRNA.
49. Wethmar, K., Smink, J. J. & Leutz, A. Upstream open reading frames: molecular switches in (patho) physiology. *Bioessays* **32**, 885–893 (2010).
50. Beckmann, K., Grskovic, M., Gebauer, F. & Hentze, M. W. A dual inhibitory mechanism restricts *msl-2* mRNA translation for dosage compensation in *Drosophila*. *Cell* **122**, 529–540 (2005).
51. Duncan, K. E., Strein, C. & Hentze, M. W. The SXL-UNR corepressor complex uses a PABP-mediated mechanism to inhibit ribosome recruitment to *msl-2* mRNA. *Mol. Cell* **36**, 571–582 (2009).
52. Medenbach, J., Seiler, M. & Hentze, M. W. Translational control via protein-regulated upstream open reading frames. *Cell* **145**, 902–913 (2011).
53. Naarmann, I. S. *et al.* DDX6 recruits translational silenced human reticulocyte 15-lipoxygenase mRNA to RNP granules. *RNA* **16**, 2189–2204 (2010).
54. Johnstone, O. & Lasko, P. Interaction with eIF5B is essential for Vasa function during development. *Development* **131**, 4167–4178 (2004).
55. Liu, N., Han, H. & Lasko, P. Vasa promotes *Drosophila* germline stem cell differentiation by activating *mei-P26* translation by directly interacting with a (U)-rich motif in its 3′ UTR. *Genes Dev.* **23**, 2742–2752 (2009).
56. Stefani, G., Fraser, C. E., Darnell, J. C. & Darnell, R. B. Fragile X mental retardation protein is associated with translating polyribosomes in neuronal cells. *J. Neurosci.* **24**, 7272–7276 (2004).
57. Darnell, J. C. *et al.* FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* **146**, 247–261 (2011).
This study identifies 842 potential mRNA targets for FMRP and provides evidence that FMRP stalls ribosomes and inhibits elongation.
58. Balagopal, V. & Parker, R. Stm1 modulates translation after 80S formation in *Saccharomyces cerevisiae*. *RNA* **17**, 835–842 (2011).
59. Ryazanov, A. G. & Davydova, E. K. Mechanism of elongation factor 2 (EF-2) inactivation upon phosphorylation. Phosphorylated EF-2 is unable to catalyze translocation. *FEBS Lett.* **251**, 187–190 (1989).
60. Park, S. *et al.* Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron* **59**, 70–83 (2008).
61. Weatherill, D. B. *et al.* Compartment-specific, differential regulation of eukaryotic elongation factor 2 and its kinase within *Aplysia* sensory neurons. *J. Neurochem.* **117**, 841–855 (2011).
62. Majmundar, A. J., Wong, W. J. & Simon, M. C. Hypoxia-inducible factors and the response to hypoxic stress. *Mol. Cell* **40**, 294–309 (2010).
63. Chen, P. J. & Huang, Y. S. CPEB2-eEF2 interaction impedes HIF-1α RNA translation. *EMBO J.* **31**, 959–971 (2011).
64. Hussey, G. S. *et al.* Identification of an mRNP complex regulating tumorigenesis at the translational elongation step. *Mol. Cell* **41**, 419–431 (2011).
65. Friend, K. *et al.* A conserved PUF-Ago-eEF1A complex attenuates translation elongation. *Nature Struct. Mol. Biol.* **19**, 176–183 (2012).
66. Takagi, M., Absalon, M. J., McLure, K. G. & Kastan, M. B. Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. *Cell* **123**, 49–63 (2005).
67. Chen, J. & Kastan, M. B. 5′–3′-UTR interactions regulate p53 mRNA translation and provide a target for modulating p53 induction after DNA damage. *Genes Dev.* **24**, 2146–2156 (2010).
68. Lee, J. T. & Gu, W. The multiple levels of regulation by p53 ubiquitination. *Cell Death Differ.* **17**, 86–92 (2010).
69. Ofir-Rosenfeld, Y., Boggs, K., Michael, D., Kastan, M. B. & Oren, M. Mdm2 regulates p53 mRNA translation through inhibitory interactions with ribosomal protein L26. *Mol. Cell* **32**, 180–189 (2008).
70. McGowan, K. A. *et al.* Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. *Nature Genet.* **40**, 963–970 (2008).
71. Barkic, M. *et al.* The p53 tumor suppressor causes congenital malformations in RPL24-deficient mice and promotes their survival. *Mol. Cell Biol.* **29**, 2489–2504 (2009).
72. Cmejla, R., Cmejlova, J., Handrkova, H., Petrak, J. & Pospisilova, D. Ribosomal protein S17 gene (RPS17) is mutated in Diamond–Blackfan anemia. *Hum. Mutat.* **28**, 1178–1182 (2007).
73. Draptchinskaia, N. *et al.* The gene encoding ribosomal protein S19 is mutated in Diamond–Blackfan anaemia. *Nature Genet.* **21**, 169–175 (1999).
74. Farrar, J. E. *et al.* Abnormalities of the large ribosomal subunit protein, RPL35a, in Diamond–Blackfan anemia. *Blood* **112**, 1582–1592 (2008).
75. Gazda, H. T. *et al.* Ribosomal protein S24 gene is mutated in Diamond–Blackfan anemia. *Am. J. Hum. Genet.* **79**, 1110–1118 (2006).
76. Gazda, H. T. *et al.* Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond–Blackfan anemia patients. *Am. J. Hum. Genet.* **83**, 769–780 (2008).
77. Horos, R. *et al.* Ribosomal deficiencies in Diamond–Blackfan anemia impair translation of transcripts essential for differentiation of murine and human erythroblasts. *Blood* **119**, 262–272 (2012).
78. Amsterdam, A. *et al.* Many ribosomal protein genes are cancer genes in zebrafish. *PLoS Biol.* **2**, e139 (2004).
79. Duan, J. *et al.* Knockdown of ribosomal protein S7 causes developmental abnormalities via p53 dependent and independent pathways in zebrafish. *Int. J. Biochem. Cell Biol.* **43**, 1218–1227 (2011).
80. Kondrashov, N. *et al.* Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell* **145**, 383–397 (2011).
81. Jack, K. *et al.* rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. *Mol. Cell* **44**, 660–666 (2011).
82. Bellodi, C., Kopmar, N. & Ruggero, D. Deregulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita. *EMBO J.* **29**, 1865–1876 (2010).
83. Bellodi, C. *et al.* Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis. *Cancer Res.* **70**, 6026–6035 (2010).
84. Higa-Nakamine, S. *et al.* Loss of ribosomal RNA modification causes developmental defects in zebrafish. *Nucleic Acids Res.* **40**, 391–398 (2012).
85. Anderson, P. & Kedersha, N. RNA granules: post-transcriptional and epigenetic modulators of gene expression. *Nature Rev. Mol. Cell Biol.* **10**, 430–436 (2009).
86. Thomas, M. G., Loschi, M., Desbats, M. A. & Boccaccio, G. L. RNA granules: the good, the bad and the ugly. *Cell Signal* **23**, 324–334 (2011).
87. Besse, F., Lopez de Quinto, S., Marchand, V., Trucco, A. & Ephrussi, A. *Drosophila* PTB promotes formation of high-order RNP particles and represses *oskar* translation. *Genes Dev.* **23**, 195–207 (2009).
88. Sawicka, K., Bushell, M., Spriggs, K. A. & Willis, A. E. Polypyrimidine-tract-binding protein: a multifunctional RNA-binding protein. *Biochem. Soc. Trans.* **36**, 641–647 (2008).
89. Snee, M. J. & Macdonald, P. M. Dynamic organization and plasticity of sponge bodies. *Dev. Dyn.* **238**, 918–930 (2009).
90. Knoch, K. P. *et al.* Polypyrimidine tract-binding protein promotes insulin secretory granule biogenesis. *Nature Cell Biol.* **6**, 207–214 (2004).

91. Martin, K. C. & Ephrussi, A. mRNA localization: gene expression in the spatial dimension. *Cell* **136**, 719–730 (2009).
92. Kugler, J. M. & Lasko, P. Localization, anchoring and translational control of *oskar, gurken, bicoid* and *nanos* mRNA during *Drosophila* oogenesis. *Fly* **3**, 15–28 (2009).
93. Mili, S., Moissoglu, K. & Macara, I. G. Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions. *Nature* **453**, 115–119 (2008).
94. Sutton, M. A. & Schuman, E. M. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127**, 49–58 (2006).
95. Doyle, M. & Kiebler, M. A. Mechanisms of dendritic mRNA transport and its role in synaptic tagging. *EMBO J.* **30**, 3540–3552 (2011).
96. Navarro, C., Puthalakath, H., Adams, J. M., Strasser, A. & Lehmann, R. Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate. *Nature Cell Biol.* **6**, 427–435 (2004).
97. Dienstbier, M., Boehl, F., Li, X. & Bullock, S. L. Egalitarian is a selective RNA-binding protein linking mRNA localization signals to the dynein motor. *Genes Dev.* **23**, 1546–1558 (2009)
This study provides a detailed and complete mechanism for dynein-mediated mRNA localization in *D. melanogaster* blastoderm cells.
98. Hoogenraad, C. C. *et al.* Mammalian Golgi-associated Bicaudal-D2 functions in the dynein-dynactin pathway by interacting with these complexes. *EMBO J.* **20**, 4041–4054 (2001).
99. Hoogenraad, C. C. *et al.* Bicaudal D induces selective dynein-mediated microtubule minus end-directed transport. *EMBO J.* **22**, 6004–6015 (2003).
100. Hachet, O. & Ephrussi, A. Splicing of *oskar* RNA in the nucleus is coupled to its cytoplasmic localization. *Nature* **428**, 959–963 (2004).
101. Jambor, H., Brunel, C. & Ephrussi, A. Dimerization of *oskar* 3′ UTRs promotes hitchhiking for RNA localization in the *Drosophila* oocyte. *RNA* **17**, 2049–2057 (2011).
102. Sinsimer, K. S., Jain, R. A., Chatterjee, S. & Gavis, E. R. A late phase of germ plasm accumulation during *Drosophila* oogenesis requires *lost* and *rumpelstiltskin*. *Development* **138**, 3431–3440 (2011).
103. Tanaka, T., Kato, Y., Matsuda, K., Hanyu-Nakamura, K. & Nakamura, A. *Drosophila* Mon2 couples Oskar-induced endocytosis with actin remodeling for cortical anchorage of the germ plasm. *Development* **138**, 2523–2532 (2011).
104. Vanzo, N., Oprins, A., Xanthakis, D., Ephrussi, A. & Rabouille, C. Stimulation of endocytosis and actin dynamics by Oskar polarizes the *Drosophila* oocyte. *Dev. Cell* **12**, 543–555 (2007).
105. Zimyanin, V. L. *et al. In vivo* imaging of *oskar* mRNA transport reveals the mechanism of posterior localization. *Cell* **134**, 843–853 (2008).
106. Chao, J. A. *et al.* ZBP1 recognition of β-actin zipcode induces RNA looping. *Genes Dev.* **24**, 148–158 (2010).
107. Shen, Z., St-Denis, A. & Chartrand, P. Cotranscriptional recruitment of She2p by RNA pol II elongation factor Spt4-Spt5/DSIF promotes mRNA localization to the yeast bud. *Genes Dev.* **24**, 1914–1926 (2010).
108. Chung, S. & Takizawa, P. A. Multiple Myo4 motors enhance *ASH1* mRNA transport in *Saccharomyces cerevisiae*. *J. Cell Biol.* **189**, 755–767 (2010).
109. Muller, M. *et al.* A cytoplasmic complex mediates specific mRNA recognition and localization in yeast. *PLoS Biol.* **9**, e1000611 (2011).
110. Ingolia, N. T., Ghaemmaghami, S., Newman, J. R. & Weissman, J. S. Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science* **324**, 218–223 (2009).
This article reports a technology that promises to enable precise and comprehensive studies of translation in living cells.
111. Oeffinger, M. *et al.* Comprehensive analysis of diverse ribonucleoprotein complexes. *Nature Meth.* **4**, 951–956 (2007).
112. Tadros, W. & Lipshitz, H. D. The maternal-to-zygotic transition: a play in two acts. *Development* **136**, 3033–3042 (2009).
113. Lasko, P. Posttranscriptional regulation in *Drosophila* oocytes and early embryos. *Wiley Interdiscip. Rev. RNA* **2**, 408–416 (2011).
114. Piccioni, F., Zappavigna, V. & Verrotti, A. C. A cup full of functions. *RNA Biol.* **2**, 125–128 (2005).
115. Chekulaeva, M., Hentze, M. W. & Ephrussi, A. Bruno acts as a dual repressor of *oskar* translation, promoting mRNA oligomerization and formation of silencing particles. *Cell* **124**, 521–533 (2006).
116. Zappavigna, V., Piccioni, F., Villaescusa, J. C. & Verrotti, A. C. Cup is a nucleocytoplasmic shuttling protein that interacts with the eukaryotic translation initiation factor 4E to modulate *Drosophila* ovary development. *Proc. Natl Acad. Sci. USA* **101**, 14800–14805 (2004).
117. Cencic, R. *et al.* Reversing chemoresistance by small molecule inhibition of the translation initiation complex eIF4F. *Proc. Natl Acad. Sci. USA* **108**, 1046–1051 (2011).
118. Imai, Y. *et al.* Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in *Drosophila*. *EMBO J.* **27**, 2432–2443 (2008).
119. Yang, Y. *et al.* Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. *Proc. Natl Acad. Sci. USA* **103**, 10793–10798 (2006).
120. Tain, L. S. *et al.* Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. *Nature Neurosci.* **12**, 1129–1135 (2009).
121. De Rubeis, S. & Bagni, C. Regulation of molecular pathways in the fragile X syndrome: insights into autism spectrum disorders. *J. Neurodev. Disord.* **3**, 257–269 (2011).
122. Sahoo, T. *et al.* Prader–Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. *Nature Genet.* **40**, 719–721 (2008).

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
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