POST-TRANSCRIPTIONAL CONTROL

Translational control of localized mRNAs: restricting protein synthesis in space and time

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Abstract | As highlighted by recent genome-wide analyses in diverse organisms and cell types, subcellular targeting of mRNAs has emerged as a major mechanism for cells to establish functionally distinct compartments and structures. For protein synthesis to be spatially restricted, translation of localizing mRNAs is silenced during their transport and is activated when they reach their final destination. Such a precise translation pattern is controlled by repressors, which are specifically recruited to transport ribonucleoprotein particles and block translation at different steps. Functional studies have revealed that the inactivation of these repressors, either by pre-localized proteins or in response to conserved signalling pathways, triggers local protein synthesis.

Ribonucleoprotein complex
A multimolecular complex that is composed of mRNAs and associated *trans*-acting factors (proteins or non-coding RNAs).

Over the past 20 years, mRNA targeting coupled to local translation has been recognized as a powerful means to spatially restrict the synthesis of specific proteins in cells. In particular, for proteins that can be deleterious to the cell when expressed ectopically, the ability to precisely restrict their synthesis has obvious importance[1]. Furthermore, transcript localization allows for the superimposition of multiple layers of control. The prevalence of this phenomenon has become apparent with the advent of genome-wide analyses in diverse organisms and cell types (Supplementary information S1 (table)). These studies have revealed that a vast number of mRNAs display specific subcellular localizations (for example, apical–basal, membrane associated, centrosome, spindle pole or astral-microtubule associated), which indicates that mRNA localization is an important mechanism that is used by cells to establish functionally distinct compartments and structures[2].

What is the purpose of localizing mRNAs? Answers can be found in diverse types of somatic cells, most of which display some form of polarization and functional compartmentalization. For instance, whereas much is known about the composition of the complexes that establish and maintain epithelial cell polarity, an outstanding issue is that of how their individual protein components, many of them cytoplasmic, achieve their apical or basolateral membrane localization. Recent genetic studies have revealed that mRNAs that encode two key polarity regulators, Stardust and Crumbs (both are components of the conserved apical Crumbs–Stardust–PATJ complex), are localized apically, thus contributing to the establishment of epithelial cell polarity[3,4]. Localized mRNAs also regulate directed cell migration. In fibroblasts, the localization of β-actin mRNA coupled with its translation at the leading edge promotes local cytoskeletal assembly, cell polarization and directional movement[5]. Similarly, during neuronal development, axonal growth cones are guided by external cues that induce local synthesis of cytoskeleton regulators[6]. Semaphorin-3A, for example, provokes growth cone collapse, which triggers local translation of axonally targeted *RHOA* mRNA[7].

In differentiated neurons, up to hundreds of mRNAs are dendritically enriched, as estimated from primary cultures (Supplementary information S1 (table)). Local and specific translation of a subset of these mRNAs can allow rapid and synapse-restricted responses to neuronal stimulation[8]. Segregation of cell fate and embryonic polarity determinants is also frequently achieved by mRNA localization coupled to local translation[9–11]. Among the best-studied examples is *ASH1* mRNA localization to the tip of the daughter cell in the budding yeast *Saccharomyces cerevisiae* (FIG. 1). Localization-dependent translational activation of *ASH1* mRNA, which encodes a repressor of mating-type switching, ensures its restriction to the daughter cell and thus the generation of two cells of distinct types, a prerequisite for mating[11,12].

Localizing mRNAs are packaged into ribonucleoprotein complexes (RNP complexes) that engage with cytoskeletal motors for directed transport along cytoskeletal tracks (BOX 1) and ensure their translational

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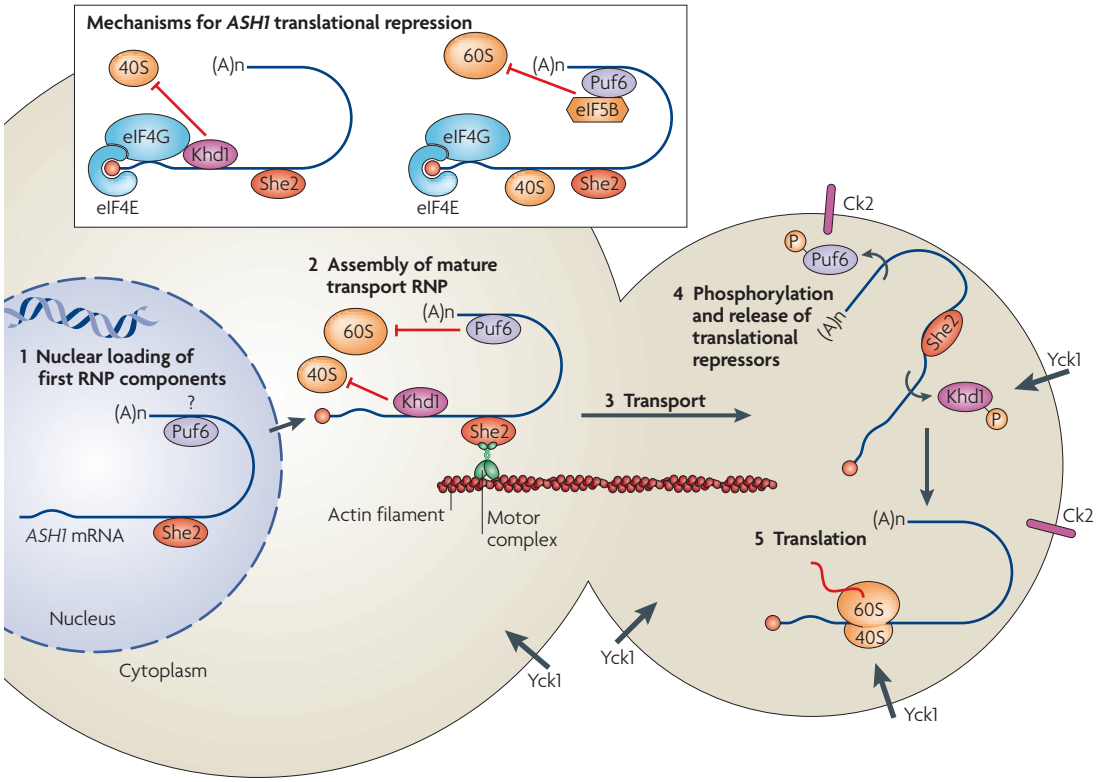


Figure 1 | **Spatial translational activation of *ASH1* mRNA in budding yeast.** *Trans*-acting factors, such as She2, first associate with *ASH1* mRNA in the nucleus (step 1), and are subsequently exported together with the mRNA to the cytoplasm. A mature transport ribonucleoprotein particle (RNP) is then assembled (step 2) by further recruitment of motor proteins and translational repressors (Khd1 (also known as Hek2) and pumilio-homology domain family-6 (Puf6)). Note that Puf6 strongly accumulates in the nucleus but has not been shown to associate with the mRNA in this compartment. During transport along actin filaments (step 3), *ASH1* mRNA translation initiation is blocked by two complementary mechanisms (inset) that prevent assembly of the eukaryotic translation initiation factor-4F (eIF4F) complex and recruitment of the 40S ribosomal subunit (Khd1-mediated mechanism; left), and prevent recruitment of the 60S ribosomal subunit (Puf6-mediated mechanism; right). After reaching the bud tip, *ASH1* RNP contacts the membrane-associated kinases Yck1 (type I casein kinase) and casein kinase-II (Ck2). Phosphorylation of Khd1 and Puf6 by Yck1 and Ck2, respectively, (step 4) induces their release from the complex, and leads to translational activation of *ASH1* mRNA (step 5). (A)n, polyadenine.

silencing. The precise composition of these complexes is dictated by the combination of *cis*-regulatory elements that are present on the mRNA and recognized by specific *trans*-acting factors. Among these factors, conserved RNA-binding proteins have been shown to control both targeting of the mRNA and translational repression, thereby providing a molecular link between these two processes[13–16]. Their inactivation at the final subcellular destination releases the mRNA from the transport machinery and allows translational activation. Depending on the cell type, mRNAs are either translationally derepressed upon arrival at their destination, or are maintained in a repressed state until specific signals lead to their activation.

Here, we provide a general overview of the composition and maturation of transport RNPs. We subsequently describe the main steps of translation that are targeted by translational repressors. The final two sections deal with the mechanisms that lead to spatially and temporally restricted activation of translation. In these sections, signals and their effects on downstream targets are mentioned and the question of specificity is discussed.

Processing body
(P body). A cytoplasmic site for mRNA degradation and the storage of translationally silenced mRNAs.

Assembling a silenced RNP

Following export to the cell cytoplasm, the mRNAs to be localized are specifically recognized by the cellular transport machinery and must be sequestered from the translational machinery until they reach their destination. Such precise sorting is achieved through the recognition of RNA *cis*-regulatory elements by *trans*-acting factors and through the subsequent assembly of RNP complexes of unique composition and structure. These complexes start to assemble co-transcriptionally in the nucleus, but undergo dynamic remodelling at different steps[17].

Composition of transport RNP complexes. Systematic proteomics analyses of RNA granule components have revealed that these complexes contain a large number of associated proteins, including RNA-binding proteins that regulate both mRNA transport and translation[18–20]. Although transport RNPs might share components with processing bodies (P bodies) — general cytoplasmic sites for translational silencing — they correspond to distinct and specific structures (BOX 2). Furthermore, whereas some conserved RNA-binding proteins are present in

Box 1 | **mRNA transport mechanisms**

Asymmetric distribution of mRNAs in a cell can be achieved through different mechanisms, including localized protection from degradation (as best shown for the *Drosophila melanogaster heat-shock protein-83* (*Hsp83*) mRNA[105]) or passive diffusion coupled with local entrapment (as shown for *D. melanogaster nanos* mRNA[106] and *Xenopus laevis Xcat2* mRNA[107]). Active and directed transport of mRNAs is the best-characterized and most commonly used mechanism. This is a multistep process that involves: first, recognition of so-called 'localization elements' that are present in the mRNA by *trans*-acting factors; second, recruitment of motor protein complexes; third, directed transport along cytoskeletal tracks; and fourth, mRNA anchoring at the final destination.

It has been shown that a few short (20–60 nucleotide) localization elements are necessary and sufficient for mRNA targeting, and are recognized by a limited number of RNA-binding proteins. However, most of the localization elements described so far are more complex and/or contain redundant, as well as complementary, sequences that ensure robustness and flexibility. Once RNA-binding proteins are specifically bound to their target mRNAs, they help to recruit motor proteins and connect the mRNA to cytoskeletal tracks. Depending on the cell type and on the mRNA, these tracks can be microfilaments (as described for yeast *ASH1* mRNA) or microtubules (as shown for *D. melanogaster oskar* mRNA and *X. laevis Vg1* mRNA). The associated motor proteins are myosins (for actin-based movement) or kinesins and/or dyneins (for microtubule plus-end- and minus-end-directed movement, respectively)[108]. Importantly, localization elements not only dictate the type of motor protein that is recruited to the mRNA, but also regulate its transport kinetics[109]. By controlling both the number of motor molecules bound and the balance between these motors, RNA-binding proteins have been shown to modulate both the velocity and the frequency of movement of transport ribonucleoprotein particles[110].

Once transported, mRNAs must be stably maintained at their final subcellular location. Strikingly, the actin cytoskeleton has been shown in various systems to control this anchoring step. Recent data have also suggested that motor proteins might also be involved in this process[111].

different types of transport RNPs[18,19,21,22], and might therefore form a core module regulating the assembly and the translational state of various transport complexes, some translational repressors bind to unique sets of target mRNAs through the recognition of specific sequences. These *cis*-regulatory sequences (or motifs) are generally found in the 3′ untranslated region (UTR) of targeted mRNAs[23], but can also be located in 5′ UTR[24] or coding[25] regions.

As revealed by biochemical purifications[18,19] and co-immunostainings[26–28], transport RNPs also contain components of the translational machinery, including ribosomal elements. Whether these components are assembled into functional ribosomes is still unclear[18,28–30]. In addition to protein factors, localizing complexes seem to include small non-coding RNAs that inhibit translation of their associated target mRNAs, such as the non-coding RNA BC1 (REF. 31) and the microRNA miR-134 (REF. 32). A more systematic analysis is now needed to determine the number and diversity of non-coding RNAs that are present in transport RNPs.

Another open question is whether mRNAs are transported and regulated as single molecules or whether they are co-assembled into multimolecular transport units. The co-assembly hypothesis has received support from biochemical and genetic analyses that show that *Drosophila melanogaster* *oskar* mRNA forms multimers *in vitro* and *in vivo*[30], and can hitchhike on other *oskar* molecules *in vivo*[33]. Interestingly, recent imaging analyses, in which distinct mRNAs have been expressed and differentially tagged *in vivo* in yeast[34] or have been co-injected in cultured oligodendrocytes[35], further suggest that several RNA species that are localized by the same transport machinery might also be co-transported in common RNPs. Formation of higher-order RNP particles that contain several mRNA molecules and associated factors (also called multiplexing) might therefore be a mechanism used by different cell types to efficiently coordinate gene expression at specific cytoplasmic sites.

Importance of RNP nuclear history. Recent studies have shown that localizing mRNAs start to assemble into RNPs in the nucleus, and that their cytoplasmic fate is dictated by factors that are recruited in this compartment, as well as by nuclear processing events[36]. For example, splicing of *oskar* mRNA at the first intron is required for its transport to the posterior pole of *D. melanogaster* oocytes[33]. Interestingly, various translational regulators that are found in transport-RNP complexes are shuttling proteins that contain nuclear localization signals and accumulate at least transiently in the nucleus[37–43]. Consistent with an association of these regulatory proteins with their target mRNAs in the nucleus, the translational repressor zip-code binding protein-1 (ZBP1) has been shown to associate co-transcriptionally with β-actin mRNA in mammalian cell lines and in chicken fibroblasts[15,44,45]. Although the functional requirement for nuclear binding remains to be tested, it might allow translational blockage at the source and prevent premature initiation of translation following export into the cytoplasm.

Overall, only a few studies have functionally tested the biological importance of the nuclear binding of translational repressors. In yeast, nucleolar translocation of the adaptor protein She2 might be required for translational repression of *ASH1* mRNA[37]. By contrast, we have shown that a strictly cytoplasmic form of the *D. melanogaster* nucleocytoplasmic shuttling protein PTB (also known as HEPH), a repressor of *oskar* translation, has the capacity to associate with *oskar* mRNA and block its translation (F.B., S. Lopez de Quinto and A.E., unpublished observations). Further functional studies are therefore required to determine, for different mRNAs and in different systems, whether nuclear recruitment of repressors is essential for subsequent translational silencing.

Localizing mRNAs are translationally repressed. It is generally thought that localizing mRNAs are translationally silenced during their transport. Although difficult to show convincingly, this assumption is supported by several lines of evidence. First, proteins that are encoded by localized mRNAs accumulate specifically at the site of their final destination, and reporter constructs that recapitulate their translational control (BOX 3) are activated at these sites. Second, translational repressors are associated with transported mRNAs and, in some cases, their loss of function has been shown to induce ectopic protein production[22,39,46,47]. Third, components of the exon junction complex, which is deposited on mRNAs following splicing and removed after their translation[48],

Exon junction complex
Following splicing, this multiprotein complex is deposited on RNAs 20–24 nucleotides upstream of exonic splice donor sites.

Box 2 | Transport RNPs and P bodies: how similar are they?

Processing bodies (P bodies) have been identified in different eukaryotic cell types as cytoplasmic sites for RNA degradation and transient storage of translationally repressed mRNAs[112]. They are characterized by the presence of translational repressors as well as enzymes and cofactors that promote mRNA decay (such as the decapping enzymes DCP1 and DCP2), but seem to lack ribosomal proteins[112]. Colocalization experiments in *Drosophila melanogaster* ovaries and embryos have suggested that transport ribonucleoprotein particles (RNPs) might share components with P bodies[63,113,114]. Furthermore, Barbee *et al.*[115] have proposed that neuronal RNPs are similar in structure and function to cytoplasmic P bodies based on co-immunostainings done on fixed *D. melanogaster* cultured neurons. A recent quantitative time-lapse microscopy analysis in mammalian neurons challenged this idea, however, and showed that localizing RNPs (labelled by the transport factor Staufen) and P bodies (labelled by DCP1) are distinct structures, the interaction of which is transient and dynamically controlled by neuronal activity[116]. Moreover, transport RNPs and P bodies seem to only partially overlap in composition, as revealed by the lack of colocalization between the P-body markers DCP2 and Pacman (*Saccharomyces cerevisiae* Xnr1) with *oskar* mRNA in *D. melanogaster* oocytes[117]. Further quantitative and systematic live-imaging studies are required to analyse the dynamic composition of transport RNPs as well as their interplay with other cytoplasmic RNA granules.

Polysomal fraction
A heavy fraction of a sucrose gradient that is enriched in polysomes and mRNAs that are undergoing translation.

are associated with transport RNPs in different systems[49–51], which further indicates that mRNAs are repressed while being transported. Finally, localizing mRNAs seem to co-sediment poorly with fractions that contain actively translated mRNAs (polysomal fractions)[28,30,52], although this observation is controversial and might depend on the transported mRNA[29,53].

Box 3 | Tools to visualize local translation *in vivo*

Fluorescent reporters. Reporters that consist of the regulatory region of a locally translated RNA fused to the coding sequence of an engineered fluorescent protein can be used to analyse spatio-temporal patterns of protein synthesis in living cells. Knowledge of the *cis*-regulatory motifs that recapitulate translational control of the endogenous mRNAs is a prerequisite. Furthermore, both the folding rate and the diffusion kinetics of the synthesized fluorescent protein must be taken into account when interpreting the data.

myr-dGFP–untranslated region (UTR) reporters encode a membrane-anchored destabilized green fluorescent protein (myr-dGFP) with reduced half-life and diffusion capacity. They were initially developed to discriminate between dendritically and somatically produced proteins in cultured neurons[118].

Kaede–UTR reporters encode a protein, kaede, that has a fluorescence that is converted from green to red following ultraviolet irradiation. After photoconversion, sites of active translation can be visualized by the appearance of newly produced green protein, as shown in developing axons[79] or mature dendrites[119] of cultured neurons.

GFP-timer–UTR reporters encode GFP-timer, the fluorescence of which changes from green to red over time. The green/red ratio can be used as a spatial indicator of the source of protein synthesis in vertebrate retinal axons[120]. However, when combined with a high diffusion rate, the slow kinetics of the green–red transition (2–3 hours) might prevent visualization of protein synthesis sites.

Biarsenical dyes. The protein produced by the mRNA of interest is tagged with a small tetra-Cys motif that is recognized by biarsenical dyes. Following binding, these dyes become fluorescent (green fluorescence for FlAsH–EDT_2; red fluorescence for ReAsH–EDT_2) and can therefore be used sequentially in pulse-chase experiments to dynamically monitor sites of protein production. This methodology has been successfully used to reveal local translation in migrating fibroblasts[121] and in cultured neurons[122].

Fluorescent puromycin conjugates. Fluorescently labelled puromycin conjugates function as structural analogues of aminoacylated tRNAs and covalently bind to any nascent protein. They have been used to detect sites of protein synthesis *in vivo* in different systems[123,124]. In contrast to the tools described above, they are general, non-mRNA-specific indicators.

Blocking translation during transport

Translational repressors, when bound to their localizing mRNAs, prevent protein synthesis by targeting different regulators of the translation process (BOX 4). Importantly, whereas translation elongation might be targeted by RNP repressors[29,53], translation initiation, which is generally rate-limiting, is the most frequently regulated step (FIG. 2a).

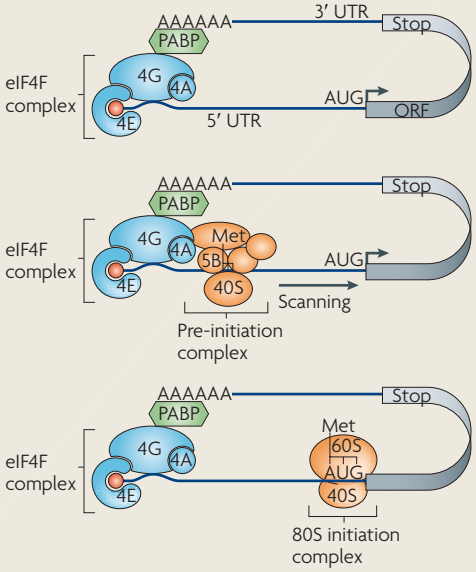
Targeting the eIF4F complex. The association of the scaffolding protein eukaryotic translation initiation factor-4G (eIF4G) to the cap-binding protein eIF4E is a step that is frequently targeted by translational repressors that are recruited to transport RNPs. Indeed, specific eIF4E-binding proteins (eIF4E-BPs) are recruited to silenced mRNAs, where they are thought to compete with eIF4G binding and thereby block the formation of the eIF4F complex, which consists of eIF4G, eIF4E and the RNA helicase eIF4A[54]. *D. melanogaster* Cup protein, for example, is an eIF4E-BP that is recruited to the *oskar* RNP by direct interaction with the 3′ UTR-bound repressor Bruno. Disruption of the Cup–eIF4E interaction leads to premature translation of localizing *oskar* mRNA[22]. In early embryos, Cup is also recruited to *nanos* mRNA by association with the 3′ UTR-associated repressor Smaug, and presumably represses translation of non-localized *nanos* mRNA by binding eIF4E to the exclusion of eIF4G[55]. Similarly, the translational repressor fragile X mental retardation protein (FMRP) is thought to help recruit the eIF4E-BP CYFIP1 (cytoplasmic FMR-interacting protein-1) to target mRNAs in mammalian neurons[56]. These models of translational repression are analogous to those proposed for the RNA-binding protein cytoplasmic polyadenylation element-binding protein (CPEB): 3′-bound CPEB associates with either of the eIF4E-BPs maskin or neuroguidin, thereby blocking the eIF4E–eIF4G interaction[54,57].

Recently, the yeast *ASH1* mRNA-binding protein Khd1 (also known as Hek2) was proposed to repress translation initiation of localizing mRNAs by a new downstream mechanism[47]. Khd1 physically interacts with the C-terminal domain of eIF4G, which is important for translational repression of *ASH1 in vivo*[47]. Although untested, Khd1 might therefore prevent recruitment of the pre-initiation complex by blocking eIF4G function.

Finally, mammalian non-coding RNA BC1 has been shown to bind to eIF4A, and might repress translation initiation of its neuron-associated mRNAs by blocking eIF4A helicase activity[58] and subsequent recruitment of the 40S ribosomal subunit (BOX 4).

Blocking 60S ribosomal subunit joining. Translational repressors can also inhibit initiation by blocking recruitment of the 60S subunit, as shown by the 3′ UTR-binding proteins ZBP1 and pumilio-homology domain family-6 (Puf6), which prevent assembly of 80S ribosomes on their respective β-actin and *ASH1* target mRNAs[15,59]. In the case of Puf6, this blockage might result from competitive binding of Puf6 to eIF5B, as the two proteins physically interact *in vitro* and their interaction domains are functionally important for *ASH1* repression *in vivo*[59].

Box 4 | **The different steps of translation**



Protein synthesis can be divided into three steps: initiation, elongation and termination. Translation initiation (see figure) requires the association of the eukaryotic translation initiation factor-4F (eIF4F) complex (which consists of the cap-binding factor eIF4E (4E in figure), the RNA helicase eIF4A (4A) and the scaffolding protein eIF4G (4G)) with the methylated guanosine cap structure at the mRNA 5′ end, and the subsequent recruitment of the 43S pre-initiation complex (which includes the 40S ribosomal subunit). Importantly, this recruitment is thought to be facilitated by the binding of eIF4G to the 3′ polyadenine tail-binding protein (PABP) and the associated circularization of mRNA molecules. After scanning along the 5′ UTR for an appropriate AUG start codon, the pre-initiation complex is then dissolved and the 60S ribosomal subunit joins the 40S subunit to form a translationally competent 80S ribosome. This process is facilitated by the factor eIF5B (5B) and initiates translation elongation.

The elongation phase is characterized by the addition of amino acids to the growing peptide and the translocation of ribosomes along the mRNA, a process that is partly controlled by the elongation factor eEF2. Finally, translation termination is associated with the release of the newly synthesized peptide and the dissociation of the ribosome from the mRNA. For a more detailed description of translation steps and regulators, see REF. 71.

Modulation of poly(A)-tail length and PABP recruitment. Modulating the polyadenine (poly(A))-tail length of mRNAs has been shown to control translational efficiency, such that short poly(A) tails are associated with a repressed state, whereas long poly(A) tails promote translation by recruitment of the poly(A)-binding protein (PABP)[60] (BOX 4). Consistent with this, translational activation of transported mRNAs is associated with an elongation of their poly(A) tail[61–63]. Mechanistically, poly(A) tail length is regulated by the opposite action of poly(A) polymerase and the deadenylation complex, and some translational repressors have been shown to control this balance. Smaug, for example, recruits the CCR4–NOT deadenylation complex onto unlocalized *nanos* mRNA by direct interaction with one of its subunits[63].

Multilayered regulatory processes. To date, most of the mechanisms proposed for mRNA-specific translational repressors involve inhibition of the cap-dependent translation initiation process. However, a recent study has suggested that translation initiation can also be controlled through a cap-independent mechanism that involves the multimerization of RNA molecules and the formation of densely packed RNPs that are inaccessible to the translational machinery[30].

As illustrated by *oskar*, *nanos* and *ASH1* mRNA, a recurrent theme is that mRNAs are regulated by multiple redundant mechanisms that target different steps and ensure precise translational control[25,64]. This is achieved by the binding of repressor proteins with multiple functions (for example, Bruno[22,30] or Smaug[55,63]), as well as by binding of multiple translational repressors (as described for *ASH1* (REFS 47,59)).

Derepression following localization

For many localizing mRNAs, translational repression is abrogated directly after arrival at the final subcellular destination. It now seems that translational derepression in response to spatial cues might commonly be mediated by decreasing the affinity of translational repressors for their target mRNAs. This can be achieved by spatially restricted phosphorylation of repressors or by competitive binding of pre-localized proteins (FIG. 2b).

Kinase-mediated release of RNA-binding repressors. Association of the RNA-binding protein ZBP1 with β-actin mRNA is required both for its transport and for its translational silencing. However, once the mRNA is localized, ZBP1 repressor function must be inactivated for β-actin translation to occur. Interestingly, ZBP1 has been shown to be a substrate of the Src kinase *in vitro* and *in vivo*, and phosphorylation by Src has been shown to decrease the binding efficiency of ZBP1 for β-actin[15]. *In vivo*, enhanced Src activity is associated with increased translation of a β-actin reporter, and expression of a non-phosphorylatable form of ZBP1 reduces the amount of locally produced β-actin protein[15]. Importantly, as revealed by *in vivo* FRET analysis, the Src–ZBP1 interaction seems to be spatially restricted to sites of β-actin translation.

Interestingly, an analogous regulatory mechanism has been described in yeast for the two *ASH1* mRNA translational repressors Khd1 and Puf6. Puf6 is phosphorylated *in vivo* and *in vitro* by casein kinase-II (Ck2), and this phosphorylation reduces the RNA-binding affinity and repression activity of Puf6 (REF. 59). Furthermore, Ck2 accumulates at the yeast bud cortex, where it colocalizes with the translated pool of *ASH1* mRNA. Similarly, Khd1 phosphorylation by the type I casein kinase Yck1 decreases the binding affinity of Khd1 for *ASH1* mRNA, and a non-phosphorylatable form of Khd1 represses translation of an *ASH1* reporter more efficiently than wild-type Khd1 (REF. 47). Finally, the Yck1–Khd1 interaction is restricted to the plasma membrane *in vivo*. Altogether, these data suggest a model in which the translational repression exerted on localizing *ASH1* is relieved once the mRNA reaches the bud tip; this is achieved by phosphorylation-induced release of Puf6 and Khd1 repressors from the complex (FIG. 1). The restricted subcellular localization of the Ck2 and Yck1 kinases might provide the specific cues that are required for spatial control of translational derepression. Importantly, additional recent reports indicate that such kinase-based regulation of RNA-binding proteins might be a mechanism that is commonly used for translational control of targeted mRNAs[24,65].

Cap
Methylated guanosine (m^7G) that is found at the 5′ end of mRNA molecules and is recognized by eukaryotic translation initiation factors, such as eIF4E.

FRET
(Fluorescence resonance energy transfer). The transfer of energy between a donor and an acceptor fluorophore in close proximity (< 10 nm). FRET is used to monitor physical interaction between two tagged proteins in living cells.

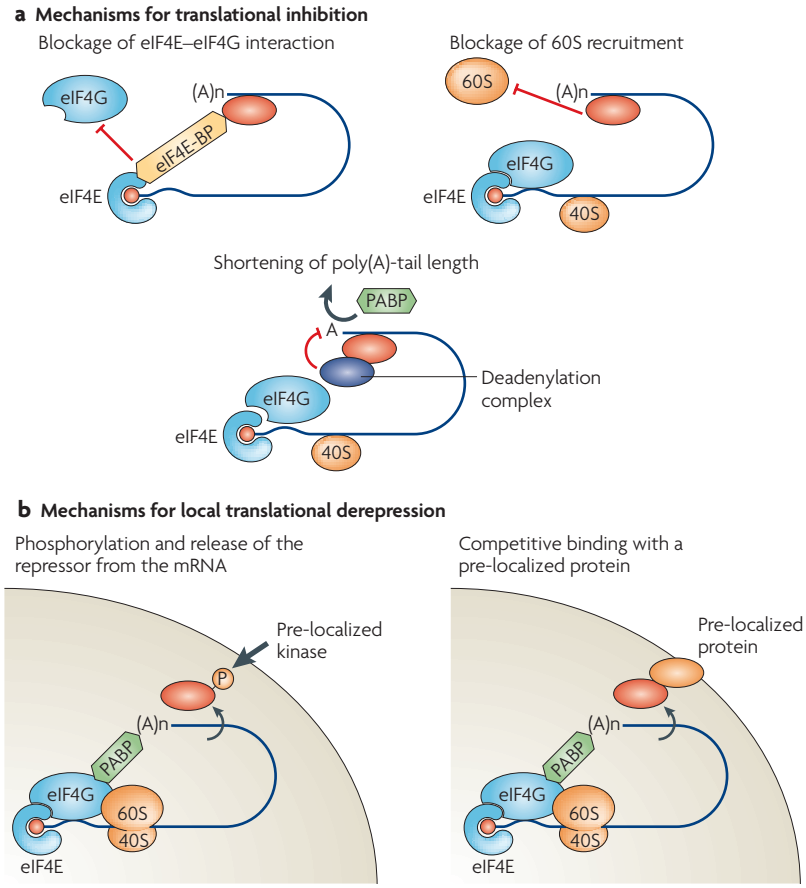


Figure 2 | **Mechanisms that control translation of localized mRNAs. a** | Established mechanisms for translational repression of transported mRNAs. Translational repressors (red) can interfere with formation of the eukaryotic translation initiation factor (eIF4F) complex when bound to localizing mRNAs by recruiting eIF4E-binding protein (eIF4E-BP), thus blocking the eIF4E–eIF4G interaction (left panel). Repressors can also block 60S ribosomal subunit joining (right panel), or decrease polyadenine (poly(A); (A)n in figure)-tail length through recruitment of deadenylation complexes (lower panel). Less well-characterized mechanisms, such as mechanisms that involve non-coding RNAs, are not shown. **b** | Mechanisms for local translational derepression. At the final destination, displacement of translational repressors from the mRNA can be triggered by phosphorylation of the repressor (left panel), or by competitive binding of pre-localized proteins (right panel). PABP, poly(A)-binding protein.

Competitive binding with locally produced proteins. Spatially restricted release of translational repressors might also be induced by the interaction of repressors with locally expressed binding partners. Oskar protein, for example, is specifically synthesized at the posterior pole of *D. melanogaster* oocytes, where *nanos* mRNA is translated. Interestingly, Oskar has been shown to interact with the translational repressor of *nanos*, Smaug, *in vitro*[66]. Furthermore, ectopic expression of Oskar leads to ectopic Nanos synthesis, and inhibits the binding of Smaug to *nanos* mRNA[63]. This suggests a model whereby posterior Oskar locally interacts with *nanos*-bound Smaug, thereby disrupting the binding of Smaug to its target mRNA and reducing translational repression of *nanos* in a spatially restricted manner. The relevance of the Smaug–Oskar interaction to *nanos* regulation in a wild-type context remains to be determined.

NMDAR
(*N*-methyl-D-aspartate receptor). An ionotropic Glu receptor that is specifically activated by the Glu analogue NMDA.

mGluR
(Metabotropic Glu receptor). A G-protein-coupled Glu receptor.

Neurotrophin
A secreted protein that prevents neuronal cell death and promotes neuronal growth.

Signal-induced translational activation

In specific cell types, targeted mRNAs are stored in a dormant state at their final destination, and their translation is activated only in response to specific external signals. This has been shown extensively in neuronal cells, in which subsets of localized mRNAs are translated in mature dendrites following synaptic activation[67] or in developing axon growth cones in response to guidance cues[6]. Signal-driven translational derepression of localized mRNAs has also been described in other systems, such as *Xenopus laevis* oocytes, in which the translation of several spindle-localized mRNAs is specifically activated following progesterone-induced meiotic maturation[14]. In neurons, external signals induce translational derepression by regulating both general components of the protein synthetic machinery and mRNA-specific repressors (see below) (FIG. 3).

General regulation of the translational machinery. In both dendrites and axon growth cones, external stimuli modulate the activity of general components of the translational machinery. For example, both eIF4E and ubiquitous eIF4E-BP are rapidly phosphorylated in vertebrate growth cones following application of guidance cues[6,68] and in mammalian dendrites in response to protocols that induce long-term changes in synaptic activity[67,69]. In the different systems studied so far, these events are controlled by two conserved receptor-coupled kinase pathways — extracellular signal-regulated kinase (ERK) signalling and mammalian target of rapamycin (mTOR) signalling[6,67] (FIG. 3). Although the exact influence of eIF4E phosphorylation is controversial, it has been associated with an overall increase in the cellular translation rate[70]. Furthermore, phosphorylation of eIF4E-BP reduces its affinity for eIF4E, thereby allowing interaction between eIF4E and eIF4G and the efficient recruitment of the translation initiation complex on capped mRNAs[71] (BOX 4).

Translation elongation of dendritically localized mRNAs is also regulated by synaptic activity. Indeed, changes in the phosphorylation status of eukaryotic elongation factor-2 (eEF2), which promotes ribosome translocation along the mRNA (BOX 4), have been observed in response to various stimuli. Notably, activation of NMDA (*N*-methyl-D-aspartate)-type Glu receptor (NMDAR) or group I metabotropic Glu receptor (mGluR) has been associated with hyperphosphorylation of eEF2 and a decrease in translation rates[72–74], whereas treatment with the neurotrophin brain-derived neurotrophic factor (BDNF) seems to reduce inhibitory eEF2 phosphorylation[75]. Furthermore, eEF2 phosphorylation is bidirectionally controlled by spontaneous and evoked excitatory transmissions in cultured hippocampal neurons, and has been proposed to mediate the switch between these two forms of synaptic activity[76].

Under physiological conditions, stimulus-driven regulation of translational machinery components is probably spatially restricted in the cell. BDNF, for example, seems to control translation in a compartment-specific manner, inducing phosphorylation of eIF4E in synaptic fractions and phosphorylation of eEF2 specifically in non-synaptic fractions[77]. Furthermore, local application of BDNF has

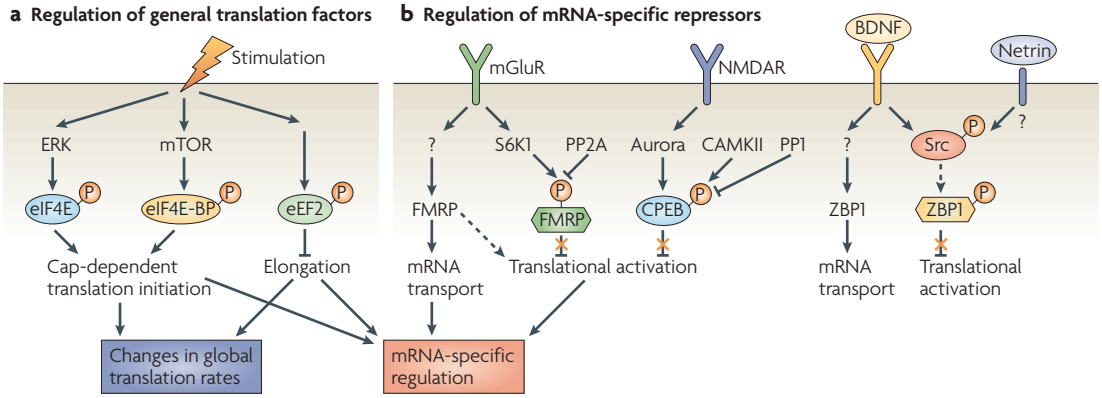


Figure 3 | **Signal-induced translational activation.** Schematic of pathways that regulate translation of mRNAs that are localized in neurons. For clarity, both input signals and regulatory networks have been simplified, and only components mentioned in the main text are shown. After stimulation, both general components of the translational machinery and mRNA-specific binding proteins are regulated to activate translation of specific sets of mRNAs. **a** | The cap-dependent eukaryotic translation initiation factor-4E (eIF4E) and eIF4E-BP are phosphorylated by the extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) signalling pathways, leading both to changes in global translation rate and mRNA-specific translational activation. In addition, phosphorylation of eukaryotic elongation factor-2 (eEF2) is controlled by various stimuli and modulates these two processes. **b** | In a complementary manner, the activity of mRNA-specific translational repressors and transport factors is differentially regulated in response to selective stimuli. It should be noted that all of the steps in the pathway that links brain-derived neurotrophic factor (BDNF) to mRNA localization and local translation, as shown here, have not been demonstrated in a single neuronal cell type. CAMKII, calcium/calmodulin-dependent Ser/Thr-protein kinase-2α; CPEB, cytoplasmic polyadenylation element-binding protein; eIF4E-BP, eIF4E-binding protein; FMRP, fragile X mental retardation protein; NMDAR, *N*-methyl-D-aspartate-type Glu receptor; mGluR, metabotropic Glu receptor; PP, protein phosphatase; S6K1, ribosomal protein S6 kinase-1; ZBP1, zip-code binding protein-1.

been shown to induce spatially restricted activation of translation initiation regulators in the dendrites of cultured neurons[78]. Finally, application of a gradient of the attractant netrin-1 leads to asymmetric accumulation of hyperphosphorylated eIF4E-BP on the proximal side of cultured axon growth cones[79]. An emerging theme in the field is that cytoskeletal elements might help to restrict translational activation to specific compartments in the cell (Supplementary information S2 (box)).

Global changes in translation rates and specificity. Surprisingly, translation of several dendritically localized mRNAs is specifically stimulated under conditions in which translation is globally downregulated[28,73,74]. Such gene-specific regulation has already been described for different mRNAs in response to changing growth conditions, and might be explained by mRNA-specific structural features and 5′ regulatory sequences[80]. For example, it is assumed that poorly initiated transcripts benefit from the increased concentration of free translation initiation factors, which is induced by a general blockage of translation elongation[73,74]. Alternatively, mRNAs that undergo cap-independent internal translation initiation might be selectively regulated in response to general modulation of cap-dependent eIF4E. Consistent with this, an activity-dependent form of plasticity in *Aplysia californica* neuroendocrine cells is associated with a switch from cap-dependent to cap-independent translation, and with a selective increase in translation of the internal ribosomal entry site-containing egg-laying hormone mRNA[81]. Interestingly, several dendritically localized mRNAs contain functional internal ribosomal entry site sequences in their 5′ UTR region, but the biological role of their internal translation initiation sites in response to synaptic stimuli has not been tested[82].

Although changes in the efficiency of the translational machinery can induce gene-specific translational regulation, additional layers of regulation are required to explain the complex translational activation pattern of localized mRNAs in neuronal cells. Indeed, signals that trigger opposite physiological responses have been shown to regulate general components of the protein synthesis machinery in a similar way. For example, although the guidance molecules netrin-1 and semaphorin-3A both induce phosphorylation of eIF4E and eIF4E-BP in *X. laevis* retinal axon growth cones, semaphorin-3A induces growth cone collapse, whereas netrin-1 induces turning towards the chemoattractant and translation of β-actin mRNA[7,79,83]. Furthermore, local application of BDNF or the mGluR agonist dihydroxyphenylglycine in the brain induces local protein synthesis-dependent long-term potentiation or long-term depression, respectively, but these opposite changes in synaptic efficacy both require phosphorylation of eIF4E and eIF4E-BPs.

Signal-specific modulation of local mRNA content. One way to achieve the local synthesis of specific sets of proteins in response to external stimuli is to modulate the composition of the pool of localized mRNAs. β-actin mRNA, for example, is present at basal levels in axon growth cones, but is further recruited to these sites following treatment with neurotrophins[84,85]. Other neuronal mRNAs, such as *ARC*, calcium/calmodulin-dependent Ser/Thr-protein kinase-2α (*CAMKIIα*), *TRKB*

Internal ribosomal entry site
(IRES). A 5′ untranslated region-located RNA sequence that recruits the 40S ribosomal subunit independently of the cap structure.

Long-term potentiation
A long-term increase in synaptic strength that is characterized by a protein synthesis-dependent late phase.

Long-term depression
A long-term decrease in synaptic activity that is characterized by a protein synthesis-dependent late phase.

or *BDNF*, are targeted to dendritic compartments in an activity-dependent manner[86–88].

Notably, synaptic activity appears to regulate the local abundance of specific mRNAs in a bidirectional manner, as shown by the mRNAs that encode the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type Glu-receptor (AMPAR) subunits 1 and 2. Indeed, both transcripts are actively transported to dendrites in response to mGluR signalling, but are depleted from this compartment following NMDAR activation[89]. Furthermore, mRNA profiling of regenerating sensory axons has revealed that the accumulation of specific mRNAs in axons can be increased or decreased in response to different ligands[90]. Remarkably, this systematic analysis shows that growth-promoting and growth-inhibiting stimuli differentially regulate the levels of individual axonal mRNAs. Although not demonstrated, specific ligands could, in principle, promote the coordinated transport of different mRNAs by activating RNA-binding proteins that recognize specific sets of mRNAs that share similar motifs. Consistent with this model, the RNA-binding protein FMRP has been shown to promote microtubule-dependent transport of dendritically localized mRNAs following mGluR stimulation[13].

Signal-specific regulation of translational repressors. In response to distinct stimuli, another way to selectively regulate the translation of subgroups of localized mRNAs is to differentially modulate the activity of mRNA-specific translational repressors. The translational regulator CPEB binds to CPE-containing mRNAs, and its phosphorylation is associated with a switch in role from translational repression to translational activation of target mRNAs. Indeed, whereas non-phosphorylated CPEB blocks translation by recruiting eIF4E-BPs, such as maskin or neuroguidin, phosphorylated CPEB promotes translation initiation by inducing poly(A)-tail elongation, recruitment of PABP and dissociation of eIF4E-BPs from cap-bound eIF4E[57,91,92]. In synaptic fractions of mammalian hippocampal neurons, Glu stimulation induces Aurora kinase-dependent phosphorylation of CPEB, which promotes poly(A)-tail elongation and translation of the CPE-containing *CAMKIIα* target mRNA[93]. In these conditions, CPEB phosphorylation depends on NMDAR signalling, as it is blocked by drugs that selectively target this receptor type, but is not affected by modulation of AMPAR and mGluR[93]. Notably, both the levels and the duration of CPEB phosphorylation can be further regulated by complementary signalling, in particular by the combined action of CAMKIIα and protein phosphatase-1 (REFS 94,95).

Consistent with specific translational repressors being targeted by specific signalling pathways, FMRP seems to be differentially required for distinct protein synthesis-dependent long-term synaptic changes in the mammalian brain. As shown in *Fmr1*-knockout mice, FMRP function is dispensable for NMDAR-mediated long-term potentiation[96], but is essential for correct mGluR-mediated long-term depression[97]. Furthermore, mGluR signalling has recently been shown to dynamically regulate FMRP phosphorylation by controlling the opposite enzymatic activities of ribosomal protein S6 kinase-I (S6K1) and protein phosphatase-2A[98]. Given that FMRP phosphorylation has been proposed to suppress mRNA target expression[98,99], mGluR-dependent protein synthesis might be triggered, at least partially, by dephosphorylation of FMRP. Whether this dephosphorylation event is linked to the release of the FMRP-associated eIF4E-BP CYFIP1 that is observed following synaptic activity remains to be tested[56].

Alleviation of microRNA-based translational repression might also be a mechanism that is used to activate translation of select target mRNAs in response to synaptic activation, although specific activation pathways that regulate this process have not been described. As shown in mammalian neurons, application of BDNF relieves miR-134-dependent translational repression of the LIM-domain kinase-1 gene[32] and might also regulate translation of other dendritically localized miR-134-binding site-containing mRNAs. In *D. melanogaster*, cholinergic activity has been proposed to induce proteasome-mediated degradation of Armitage, a component of the RNA interference complex that is involved in microRNA silencing[100]. This would inhibit microRNA-mediated repression and induce translation of mRNA targets, such as *CAMKII*. Whether the putative microRNA-binding sites that are present in the *CAMKII* 3′ UTR are indeed required for translational regulation in response to synaptic activity, however, remains to be tested.

Conclusion and perspectives

It is now clear that the precise transport and translation pattern of localized mRNAs is dictated by the combination of *cis*-acting elements present on the RNA molecule. These elements are recognized by *trans*-acting factors (either RNA-binding proteins or non-coding RNAs), the recruitment of which to the RNA contributes to the formation of an RNP of defined specificity. Bioinformatics analyses performed in yeast, flies and nematodes have revealed that 2–8% of the genomes of these organisms are predicted to encode RNA-binding proteins, each of which might have hundreds to thousands of target mRNAs[101]. Altogether, this allows for enormous combinatorial potential and thus a main challenge is to now decrypt the combinatorial code of regulatory elements and to start predicting mRNA behaviour based on primary sequence. Progress in this direction has recently been made for CPE-mediated translational control[102]. Based on their study of cyclin B1–B5 mRNAs, and taking into account three types of *cis*-acting elements, the authors could propose a highly predictive combinatorial code that determines the translational repression pattern, as well as the timing of mRNA translational activation in *X. laevis* oocytes[102]. Comparative analysis of the sequence and structure of mRNAs with similar distributions and translation profiles (Supplementary information S1 (table)), combined with the systematic identification of mRNAs associated with specific sets of *trans*-acting factors, will help to further refine regulatory motifs and further understand the codes that control RNP-complex composition and behaviour.

Finally, studying how nuclear maturation controls RNP architecture and composition should further help to understand how the nuclear history of a given

AMPAR
(α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor). An ionotropic Glu receptor that is specifically activated by the agonist AMPA.

mRNA affects its cytoplasmic fate. As recently revealed, by regulating alternative splicing and alternative usage of polyadenylation sites, nuclear factors might have a determining role in the ultimate combination of *cis*-regulatory elements present on an mRNA, and thereby control mRNA localization and translation patterns[3,103,104]. Systematic studies are now needed to determine the prevalence of these types of gene regulation.

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