# Intracellular mRNA transport and localized translation

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Abstract | Fine-tuning cellular physiology in response to intracellular and environmental cues requires precise temporal and spatial control of gene expression. High-resolution imaging technologies to detect mRNAs and their translation state have revealed that all living organisms localize mRNAs in subcellular compartments and create translation hotspots, enabling cells to tune gene expression locally. Therefore, mRNA localization is a conserved and integral part of gene expression regulation from prokaryotic to eukaryotic cells. In this Review, we discuss the mechanisms of mRNA transport and local mRNA translation across the kingdoms of life and at organellar, subcellular and multicellular resolution. We also discuss the properties of messenger ribonucleoprotein and higher order RNA granules and how they may influence mRNA transport and local protein synthesis. Finally, we summarize the technological developments that allow us to study mRNA localization and local translation through the simultaneous detection of mRNAs and proteins in single cells, mRNA and nascent protein single-molecule imaging, and bulk RNA and protein detection methods.

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Asymmetric mRNA distribution was first reported in 1983 by Jeffery et al.[1], who observed that, during the early stages of ascidian embryonic development, the mRNA encoding actin localized in the cytoplasm, where muscle-forming cells reside[1]. This observation, along with studies in *Xenopus laevis* eggs[2] and *Drosophila melanogaster*[3,4], led to the hypothesis that, during embryogenesis, specific mRNA pools could be partitioned and anchored into particular cell lineages to determine tissue differentiation. Subcellular mRNA localization was first reported in 1986 by Lawrence and Singer, who observed this phenomenon in chicken fibroblasts using in situ hybridization[5], and was subsequently shown to occur in other organisms such as *Saccharomyces cerevisiae*[6], rice plant cells[7] (BOX 1), mammalian neurons[8] and oligodendrocytes[9]. Later, mRNA localization was found to encompass a notable percentage of the transcriptome during *D. melanogaster*[10] and *X. laevis*[11] development as well as in *Escherichia coli*[12] and in mammalian tissue[13], including at the subcellular level[14], in cellular protrusions[15] and in organelles such as the endoplasmic reticulum (ER)[16]. These studies suggested that mRNA localization contributed to the post-transcriptional fine-tuning of gene expression and the control of fundamental processes such as cell migration, polarization and differentiation.

In cells, localizing mRNAs, which can be translated tens to hundreds of times in response to local stimuli, is more cost-effective than transporting individual proteins. Furthermore, localized protein synthesis may avoid the ectopic expression of a protein in an undesired compartment and regulate protein function by controlling its local concentration, its chemical environment (including its pH), and its ability to form multi-protein complexes with defined stoichiometry[17] and specific post-translational modifications[18]. Observing mRNAs at high resolution revealed that, across all species, mRNAs localize to specific compartments (for example, dendrites, axons and RNA–protein granules) and organelles (for example, ER, mitochondria and chloroplasts) to control in situ protein synthesis and local cell physiology. In addition, localization events are coordinated with the physiological status of the cell, which can influence whether mRNAs are stored in a translationally repressed state in stress granules or targeted to processing bodies (P-bodies) for degradation. mRNAs are primarily sorted via *cis*-localization elements called 'zipcodes' that, together with RNA binding proteins (RBPs), control active and passive mRNA trafficking[19]. Furthermore, non-canonical modes of mRNA transport via non-specific interactions with organelles or RNA–RNA interactions are also thought to exist[20–23].

The localization and translation of mRNAs influence cell physiology at the subcellular, cellular, tissue and organism level. At the single-cell level, mRNA trafficking dictates cell polarity, motility and differentiation

**Box 1 | mRNA localization in plants**

Although subcellular mRNA localization is well characterized in microorganisms and metazoans, less is known for plant cells. Here, we briefly summarize key examples of mRNA localization in plants, and we refer readers to specialized reviews for further details[139,251]. Studies suggest that plant cells localize mRNAs to the endoplasmic reticulum (ER), mitochondria and the chloroplast. *Oryza sativa* rice endosperm cells form a tissue inside seeds that surrounds and provides key nutrients to the germinating embryo; these cells have a peculiar pattern of mRNA localization in the ER. Two mRNAs encoding the storage proteins prolamin and glutelin localize to two separate ER compartments, the protein-body ER and the cisternal interconnecting ER, respectively[7]. Localized mRNAs encoding prolamin are translated on the ER, and the proteins are retained in the ER lumen forming an ER-derived protein body I (PBI). By contrast, mRNAs encoding glutelin localize to the adjacent *cis*-ER, and the translated proteins are further exported to the Golgi[7]. The localization of these mRNAs is independent of translation and requires specific mRNA zipcodes and RNA binding proteins (RBPs)[251–254]. Recent work showed that the mRNA encoding glutelin is transported to the cisternal ER via endosome trafficking mediated by RBP-P, RBP-L and the endosome membrane-bound protein Rab5a[255]. Besides mRNA localization to the ER, nuclear-encoded mRNAs are also transported to mitochondria. mRNA encoding the voltage-dependent ion channel (*VDAC*) localizes to mitochondria in *Arabidopsis thaliana*[256]. Owing to a *cis*-element present in the 3′ untranslated region (3′UTR), *VDAC* and other mitochondria-localized mRNAs are proposed to be transported (via an unknown mechanism) to modulate mitochondria function and number[256,257]. Finally, the mechanism for localizing RNA to chloroplasts is exploited by plant viruses for replication[251,258,259]. For instance, the negative strand of *Bamboo mosaic* virus RNA is imported into the chloroplast via a specific RNA sequence, which is a key step for viral replication[260]. Further work is required to elucidate the mechanisms controlling the transport of other mRNAs to specific subcellular locations in plants. Furthermore, the field lacks fundamental tools to investigate the mechanisms controlling localized mRNA translation. The recent description of a single-molecule fluorescence in situ hybridization (smFISH) protocol for plant cells[261] may trigger the development of novel tools aimed at elucidating localized mRNA translation in plants at high resolution.

by enabling rapid and localized responses to intracellular and extracellular signals, yet impairment of mRNA localization in single cells rarely causes severe growth phenotypes or lethality. However, in tissues or multicellular organisms, mRNA localization is crucial in homeostasis, differentiation and development. Indeed, blocking mRNA localization in the developing *D. melanogaster* embryo results in severe developmental defects[24], and mRNA localization defects in the central nervous system result in cognitive disorders[25]. Thus, to determine how mRNA localization influences high order cellular organization and functions, local gene expression should be studied from the subcellular to the multicellular level.

Although cytoplasmic mRNA localization is widespread, fundamental questions remain about its functional role and it is unclear if, and how, mRNA localization always controls local protein synthesis. In addition, improving the sensitivity of mRNA imaging technologies to study this process in intact single-cell and multicellular organisms remains challenging.

In this Review, we provide an overview of the rapidly evolving technological advances that drive research into mRNA localization and summarize evidence of mRNA localization and protein synthesis in single-cell organisms (bacteria and fungi) and in the multicellular context, for example, in tissues and whole organisms (*D. melanogaster* and *Caenorhabditis elegans*). We then describe key examples of subcellular mRNA localization in different cell types (neurons and fibroblasts) and organelles (mitochondria, ER and centrosomes), before

discussing the mechanisms governing mRNA localization and localized translation. Finally, we highlight the importance of studying the composition of mRNA granules to understand how it determines the specificity and fate of an mRNA, and we discuss the future challenges and perspectives in the field.

## Localized RNAs across kingdoms of life

Evidence of localized mRNAs and protein synthesis in single-cell and multicellular organisms has revealed that mRNA localization has a range of functions across organisms and has evolved from non-conserved and conserved mechanisms.

### Single-cell organisms

Single-cell organisms, such as bacteria and fungi, take advantage of the asymmetric distribution of mRNA and proteins to modulate gene expression and organize cellular functions. Understanding this process in single-cell organisms helps to elucidate the mechanisms that are conserved in more complex models and, from a microbiological perspective, is of both biomedical and biotechnological interest.

***RNA compartmentalization in bacteria.*** Prokaryotic cells were thought to lack intracellular mRNA localization due to their small size, the lack of membrane-bound organelles and the existence of co-transcriptional translation. However, high-resolution imaging revealed distinct compartmentalization of DNA (nucleoids), RNAs (mRNAs and small regulatory RNAs), RNA polymerases and ribosomes, providing evidence for uncoupling between transcription and translation[26–28] (FIG. 1a). Furthermore, specific mRNAs accumulate in subcellular domains where the encoded proteins reside. For instance, *cat* mRNA (encoding cytoplasmic chloramphenicol acetyltransferase) gathers in helical cytoplasmic structures[12], suggesting that the mRNA is localized where the protein is needed. Furthermore, mRNAs encoding the transmembrane transporter lactose permease (*ptsC* mRNAs)[29] or the membrane-bound lactose permease (*lacY* mRNAs)[12] are excluded from the nucleoid region and localize next to the cell membrane. Finally, mRNA granules associated with nucleoids (that is, where the protein is located) were observed for the *lacZ* mRNA (encoding β-galactosidase) both in *E. coli*[30] and *C. crescentus*[31], further suggesting that, in bacteria, mRNA localization contributes to the timing and localization of protein synthesis. Studies performed over the past 10 years show that RNA localization patterns in bacteria can be dependent on[32] or independent of[12] translation, suggesting that multiple and yet uncharacterized schemes for spatially regulating gene expression exist in prokaryotic cells.

***mRNA localization in asymmetrically dividing yeasts.*** mRNA localization has been extensively characterized in many fungal species (FIG. 1b), including in the Ascomycota *S. cerevisiae* (a model organism with biotechnological relevance) and *Candida albicans* (an opportunistic human pathogen). mRNA transport has also been characterized in the Basidiomycota plant pathogen *Ustilago maydis*[33,34].

Tens of mRNAs are localized to the bud of *S. cerevisiae* by the motor protein SWI5-dependent HO expression protein 2 (She2)–She3 complex[35]. The best characterized of these mRNAs is *ASH1*, which accumulates at the bud tip during anaphase[6,36–38]. This mRNA is transported on actin filaments in a translation-repressed state controlled by RBPs such as Pumilio homology domain family member 6 (also known as Puf6), 60S ribosomal subunit assembly/export protein LOC1 (also known as Loc1) and casein kinase I homologue 1 (also known as Khd1)[39,40]. Local activation of translation in the bud requires the phosphorylation of Khd1 and Puf6 by the bud-localized casein kinase I homologue 1 (also known as Yck1)[40] and CK2 kinase[41], respectively. The Ash1 protein is then
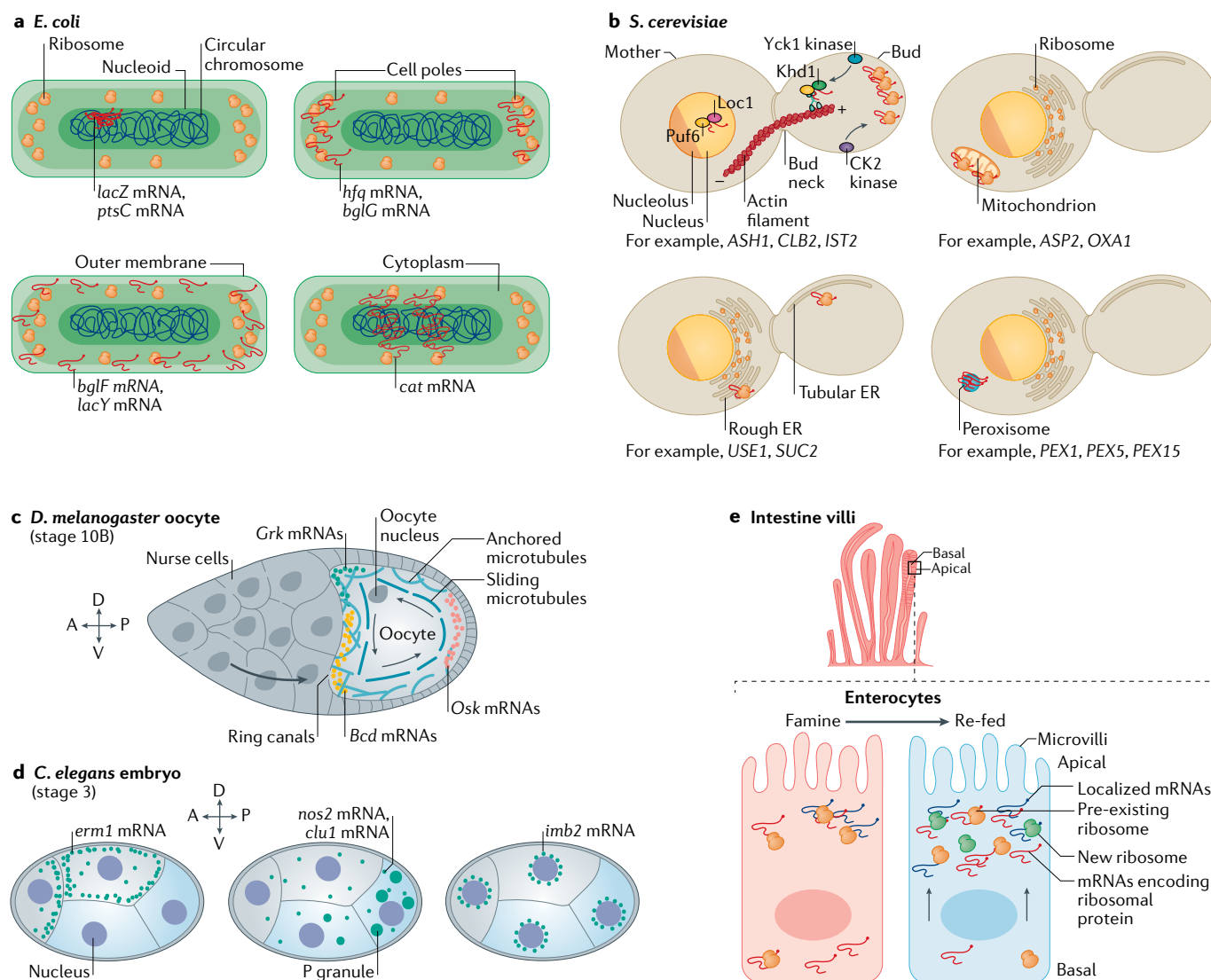


**Fig. 1 | mRNA localization and local translation in single-cell and multi-cellular organisms. a |** In prokaryotes, such as *Escherichia coli*, the cell is divided into specific sub-compartments, namely the nucleoid (where the circular DNA molecule resides), the cell poles (where ribosomes accumulate) and the outer membrane (where both ribosomes and transporters reside), to which several mRNAs have been shown to localize. *ptsC* and *lacZ* mRNAs localize to the nucleoid, *hfq* and *bglG* mRNAs localize at the cell poles, *bglF* and *lacY* mRNAs localize to the outer membrane, and *cat* mRNA has a characteristic helical distribution in the cytoplasm. **b |** In unicellular eukaryotes like *Saccharomyces cerevisiae*, mRNA is asymmetrically distributed in multiple subcellular compartments. In the growing bud, mRNAs such as *ASH1*, *CLB2* and *IST2* are actively transported on actin filaments by the She2–She3–Myo4 complex. Sequences in the 3′ untranslated region (3′UTR) of *ASTP2* and *OXA1* mRNAs localize these mRNAs to the outer mitochondrial membrane. *USE1* and *SUC2* mRNAs are localized to the endoplasmic reticulum (ER). *PEX1*, *PEX5* and *PEX15* mRNAs are found in peroxisomes. **c |** During mid-oogenesis in *Drosophila melanogaster*, the microtubule cytoskeleton of the oocyte is reorganized by cytoplasmic streaming (sliding microtubules) to localize the mRNAs that determine body plan. While *Bcd* and *Grk* mRNAs are positioned on the anterior side, *Osk* mRNAs primarily occupy the posterior side; all three mRNAs are locally translated at their respective positions. **d |** In *Caenorhabditis elegans*, maternally inherited transcripts display distinct localization patterns. Transcripts in anterior-biased cells (grey) tend to localize to the cell periphery, where the encoded protein localizes (for example, *erm1*). mRNAs enriched in posterior cells (blue), such as *nos2* and *clu1*, form clustered granules that overlap with P granules. The *imb2* mRNA localizes at the perinuclear region. **e |** In the intestine, enterocytes lining the villi are polarized cells with distinct apical and basal sides. Components of the translation machinery change their apical–basal distribution in response to nutrient availability. As mRNAs encoding ribosomal proteins move from the basal to the apical side via microtubules, the translation of mRNAs localized at the apical side is boosted to favour nutrient absorption. A, anterior; D, dorsal; P, posterior; V, ventral.

imported to the daughter nucleus, where it represses the mating-type switching programme by blocking the expression of the homothallic endonuclease[42–44]. Thus, the asymmetric localization of Ash1 protein ensures that mother and daughter cells acquire opposite mating types to be able to transition to a diploid state. Interestingly, *ASH1* mRNA is also localized in *C. albicans* hyphae in a She3-dependent manner[45]. About 40 mRNAs localize in these highly polarized and elongated cells, and the inhibition of mRNA transport impairs hyphal development with possible implications on the development and structural stability of biofilms (multicellular structures critical for fungal virulence)[34,45].

In addition to *ASH1* mRNA, our recent work demonstrated that *CLB2* mRNA, encoding the mitotic regulator B-type cyclin, is also localized to the *S. cerevisiae* bud by the She2–She3 complex and translated in situ to control mitotic entry (E.T. and R.S., unpublished work). Unlike Ash1, Clb2 (also known as G2/mitotic-specific cyclin 2) is not segregated to the daughter cell but is translocated back to the mother nucleus. This observation suggests that mRNA localization can control the temporal expression of proteins as well as their asymmetric distribution. Interestingly, orthologues of the *CLB2* mRNA are also symmetrically distributed during mouse[46], zebrafish[46] and *X. laevis*[47] oocyte development, suggesting that the localization of mRNA encoding cyclin B1 may be functionally conserved during differentiation.

mRNAs are also localized in the ER[48–50] and mitochondria[51,52] in *S. cerevisiae*. mRNAs are transported to the ER via signal recognition particle (SRP) and translation-independent pathways that require specific RBPs (for example, She2 and Puf2) and they encode both secreted and non-secreted proteins[48–50]. mRNAs can also localize to peroxisomes or cytoplasmic membrane-less granules such as P-bodies and stress granules (see below)[33]. Interestingly, mRNAs encoding translation factors (for example, *TEF1* and *YEF3*)[53] or glycolytic enzymes (for example, *PGK1*, *ENO1* and *ENO2*)[54] are found in cytoplasmic granules, suggesting that the expression of highly abundant mRNAs can be buffered by controlling their availability.

Finally, work has been conducted to elucidate mRNA transport mechanisms in the filamentous hypha *U. maydis*[33,55,56]. This research demonstrated how mRNA trafficking is intertwined with the transport of endosomes (vesicular structures also involved in the asymmetric distribution of proteins and lipids)[57]. Endosome-mediated mRNA transport requires the RBP Rrm4 (REFS[57,58]) and the *U. maydis* PAM2 protein (also called Upa1 protein)[59], which couple the mRNA and the associated ribosomes to the endosome and allow their transport on microtubules (see below). This process is important for polarized growth[57] and may promote the ability of *U. maydis* to differentiate from its single-celled form to its multicellular filamentous form when infecting plants.

### Multicellular organisms

The importance of timing gene expression by localizing mRNAs is apparent during developmental processes, such as asymmetric cell division and embryonic patterning, and studying mRNA distribution in multicellular

organisms has allowed local translation to be linked to physiological changes.

*Regulating developmental stages.* Studies in *D. melanogaster* oocytes and *C. elegans* early syncytial embryos using single-molecule fluorescence in situ hybridization[60,61] (smFISH) (Supplementary Box 1) and the MS2–MS2 coat protein system (MS2–MCP)[10,20,62] (BOX 2) have extended previous observations[24] showing that localizing mRNAs determine body axes by asymmetrically distributing specification factors of cell fate. During *D. melanogaster* oogenesis, surrounding nurse cells provide the transcriptionally quiescent oocyte with mRNAs and proteins for its development[24]. At late oogenesis, nurse cells contract, which squeezes their cytoplasm into the oocyte, depositing hundreds of mRNAs encoding patterning factors through cytoplasmic bridges known as 'ring-canals'[10,24] (FIG. 1c). Upon entering the oocyte, *Bcd* (encoding homeotic protein bicoid; also known as bicoid) and *Grk* (encoding Gurken) mRNAs are actively transported along microtubules to the anterior pole, while *Osk* mRNA (encoding Oskar), along with the RBPs Staufen and Vasa, accumulate at the posterior axis (FIG. 1c). Tagging *Twi* mRNA (the protein product of which, Twist, is the transcriptional activator of the mesodermal gene network) with the SunTag system (BOX 3) revealed that 'translation factories' localize to the basal perinuclear space of living *D. melanogaster* embryos at nuclear cleavage cycle 14 (REF.[63]). These factories, which are composed of 2–6 mRNAs, have slow diffusion dynamics, and mRNAs here are preferentially translated (see below).

In *C. elegans*, essential maternal transcripts are delivered to the germ cells to support cellular differentiation during embryogenesis (FIG. 1d). Specific transcripts asymmetrically localize in subcellular compartments, such as P-granules (for example, *nos2* and *clu1*), to the membrane (for example, *erm1*) or to the peri-nuclear region (for example, *imb2*)[64–66]. P-granules are heterogeneous RNA assemblies that segregate asymmetrically with the P-lineage during embryogenesis. smFISH demonstrated that the repression of mRNA translation is a pre-requisite for, and occurs prior to, the localization of mRNA in P-granules[66]. Thus, besides mRNA localization, the timing of local translation regulates post-transcriptional gene expression during the development of multicellular organisms.

*Tissue functionality and homeostasis in the gut and the brain.* The development of in situ transcriptomics (Supplementary Box 1) has enabled the characterization of mRNA localization patterns within native tissue, advancing our understanding of tissue architecture and functionality. Mammalian gut physiology relies on the apical–basal polarization of epithelial enterocyte cells (FIG. 1e). The apical side absorbs nutrients from the intestinal lumen while the basal side excretes them to the blood. By combining smFISH, in situ transcriptomics and proteomics, researchers demonstrated the asymmetric localization of mRNAs and proteins in the intestinal epithelium and showed that the translation machinery has a preferential apical distribution that can be enhanced upon the refeeding of fasted mice[67] (FIG. 1e).

## Box 2 | Imaging mRNA in living cells

Here, we summarize the latest methods for imaging mRNAs in live cells; methods for imaging mRNA in fixed cells are described in Supplementary Box 1. We refer the reader to specialized reviews for further details[26,248,262–265].

Several fluorescence-based methods allow mRNAs to be visualized and tracked in living cells[248,262,266], either via aptamer-based modification of the target mRNA or by detecting endogenous unmodified mRNAs. Aptamer-based mRNA labelling approaches employ RNA stem-loops derived from either bacteriophages (for example, MS2 (REFS[36,38,266,267]), PP7 (REF.[268]) or P22 N-peptide for the λ BoxB loop[269,270]) or from RNA–protein recognition motifs (for example, U1A[169,271] or Bgl bacterial anti-terminator[272]). Arrays consisting of tens of loops are commonly inserted into the 3′ untranslated region (3′UTR) of the target mRNA. Recognition by the cognate RNA binding protein (RBP) fused to a fluorescent protein or a fluorogenic tagging system (for example, HALO[273]) allows individual mRNAs to be detected in living cells (see the figure, part **a**). The MS2 and PP7 aptamers are the best-characterized tagging systems and they are well tolerated in transgenic *Drosophila melanogaster*[20,274] and in mouse lines[172,275]. They have been used for the dual labelling of the same mRNA molecule or of different mRNAs[152,276,277] to study the regulation of gene expression. Recently, fluorogenic reporters that rely on RNA aptamers mimicking the structure of GFP have been developed and they become fluorescent upon binding to conditionally fluorescent dyes. Among the best characterized of these reporters are Spinach2 (REF.[278]), Broccoli[279], Corn[280] and Mango[281,282] (see the figure, part **b**).

Live-cell imaging reporters that do not require mRNA to be modified include molecular beacons, which are oligonucleotides tagged at their 3′ and 5′ ends with a fluorophore and a quencher[283]. When the molecular beacon is not bound to the target mRNA, the fluorophore is inactive due to its close proximity to the quencher but the situation is reversed upon molecular beacon–mRNA hybridization (see the figure, part **c**). Finally, CRISPR–Cas systems such as Cas9-GFP[284], Cas9-MS2 or PP7-fusion[285] or dCas13-EGFP[286] can be exploited to target an mRNA via specific single-guide RNAs (sgRNAs) (see the figure, part **d**).

To date, stem loop aptamers remain the most widely used technique for labelling mRNAs owing to their higher single-molecule sensitivity and specificity. However, this approach has limitations for tagging highly unstable or small RNAs as it inserts a bulky sequence that can alter the lifecycle of RNA[38,287–289]. To overcome these problems, MS2 variants that are more easily degraded have been optimized for use in eukaryotic cells[38,266,290–292]. For both aptamer-based and aptamer-free methods, improvements that increase their brightness and sensitivity are required to minimize the impact of the tagging system on mRNA physiology. For the end-user, the optimal method of choice often depends on the kind of RNA and model organisms they are working with as well as the specific biological question. Further description of these single-molecule methods and their capabilities has been reviewed in REFS[248,263,266,293].
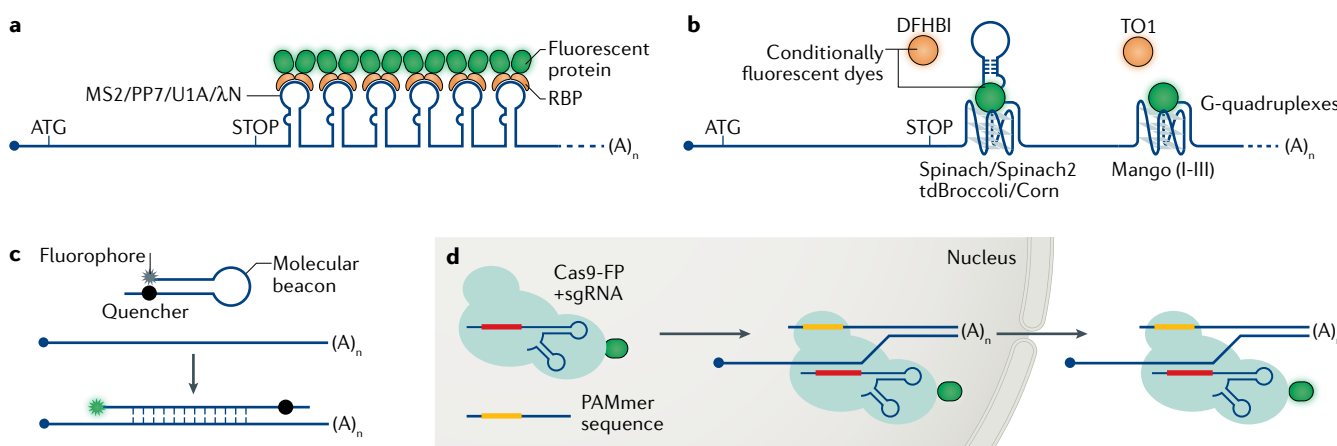


DFHBI, 3,5-difluoro-4-hydroxybenzylidene imidazolinone; FP, fluorescent protein; TO1, thiazole orange-derived fluorophore 1.

Interestingly, the apical localization of mRNAs encoding ribosomal proteins and the consequent synthesis of ribosomes on the apical side of the intestinal epithelium correlates with an increase in the translation efficiency of apically localized mRNAs, boosting nutrient absorption[67]. Thus, on top of mRNA localization, the dynamic localization of the translation machinery can fine-tune protein expression in response to environmental stimuli.

Cellular organization also determines functionality in the mammalian brain. The local transcriptome in neurons is vast and diverse (~2,500 mRNAs), as revealed by sequencing mRNAs in both the presynaptic (axonal) and postsynaptic (dendritic) sides of neurons[13], and by profiling their translation status[68,69]. The localized pool of translating mRNAs maintains local protein homeostasis to allow for physiological processes such as proper brain wiring, response to injury and activity-driven changes in synaptic strength (synaptic plasticity). Although dendritic protein synthesis has well-documented functions

in learning and memory, most studies on axonal translation have been restricted to axonal pathfinding, growth cone steering during development[70–72] and regeneration upon axonal injury[73,74]. Recent technological advancements in visualizing mRNAs and ribosomes in tissue at high resolution are now enabling us to understand the role and mechanisms of translation in the presynaptic terminals of the adult mammalian brain.

In the past few years, combinations of multi-omics approaches and imaging have been developed to identify the localized transcriptome and map the precise location of individual transcripts in the intact brain. The tissue microdissection and biochemical purification of synaptosomes has identified hundreds of localized transcripts[13,68]. A technique called expansion-FISH (ExFISH) (Supplementary Box 1), which couples physical expansion of the tissue and FISH, reduced RNA crowding and enabled high-resolution mapping of mRNAs to both presynaptic and postsynaptic compartments in the adult mammalian brain[68,75]. An omics-approach

**Aptamer**
Short RNA oligonucleotide, the sequence and structure of which is specifically recognized by an RNA binding protein (for example, the MS2 loop).

**Expansion-FISH**
(ExFISH). RNA in-situ imaging approach for fixed cells that combines sample clearing and expansion with smFISH, multiplexed smFISH or hybridization chain reaction FISH.

Box 3 | **Quantifying translation**

Approaches based on imaging, sequencing and proteomics have been recently combined to determine the site of local translation and quantify its efficiency; the imaging-based approaches are summarized here. Imaging approaches rely on two component systems that tag either different regions of the mRNA and/or the mRNA and the newly synthesized peptide. The translating RNA imaging by coat protein knock-off (TRICK) system detects the first round of translation in real time (see the figure, part **a**). PP7 and MS2 stem loops are used to label the coding sequence and the 3′ untranslated region (3′UTR), respectively; therefore, the mRNA fluoresces in two colours[152]. Ribosomes translating the coding sequence knock-off the PP7 coat protein (PCP) from PP7 stem loops, and the mRNA switches from fluorescing in two colours to fluorescing in one colour.

Both nascent chain tracking (NCT) and single-molecule imaging of nascent peptides (SINAP) systems detect nascent peptides as they exit the ribosome tunnel. An array of epitopes, cloned in frame with the open reading frame, recruit multiple copies of antibodies fused to a fluorescent protein[93,202,225,294–296]. The simultaneous detection of the peptide and the mRNA allows translation heterogeneity to be analysed, translation to be observed in subcellular compartments, and translation elongation and initiation to be quantified in real time (see the figure, part **b**).

Puromycylation-proximity ligation assay relies on the proximity of two antibodies, one that recognizes the puromycilation modification of nascent chains after puromycin treatment and another that binds to the protein of interest[297]. The secondary antibodies provide an oligonucleotide platform that is first amplified and then recognized by fluorescent oligonucleotides to generate a specific signal corresponding to the site of translation. However, recent work suggested that this system may provide inaccurate translation measurements at the subcellular scale, prompting caution (see the figure, part **c**)[298,299]. Alternatively, the translation state of an mRNA can be extrapolated by measuring the diffusion properties of fluorescently labelled single mRNAs co-moving with ribosomes (see the figure, part **d**)[128]. mRNAs loaded with polysomes have a characteristic slow diffusion and corralled movement that is used to deduce the translation state of an mRNA in living cells.

POI, protein of interest.
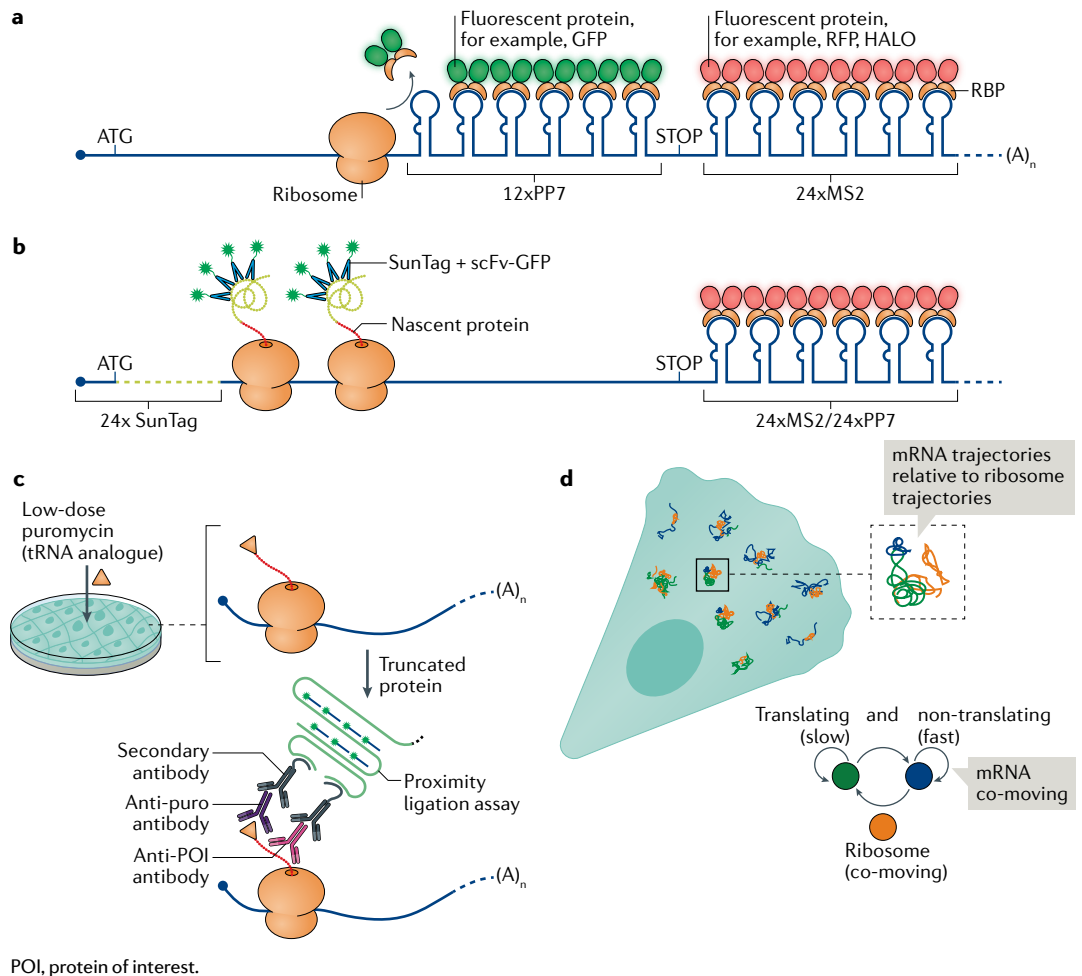
using translating ribosome affinity purification (TRAP) characterized the local axonal 'translatome' for the first time, revealing that it comprised 1,000–2,000 mRNAs in developing and mature axons of retinal ganglion cells (RGCs)[69]. Later, coupling ExFISH with stable isotope labelling by amino acids in cell culture (SILAC) revealed activity-dependent regulation of the brain translatome in a compartment-specific manner[68]. Polyribosomes have been detected in dendritic shafts and spines[76] by electron microscopy and seen to redistribute from shafts to spines in an activity-dependent manner[77]. Although challenges in visualizing polysomes in adult axons and

terminals had raised concerns over the presence of translating mRNAs in these structures, immuno-electron microscopy using genetically tagged ribosomes[69] and super-resolution methods have conclusively shown the presence of ribosomes in presynaptic terminals[68,78], supporting the occurrence of local translation in axons of the adult brain. Future studies investigating how activity alters ribosome abundance and distribution within these compartments will provide insights into the dynamic regulation of local translation. We are now beginning to elucidate the molecular mechanisms underlying translation regulation in mature axons and its importance in axonal survival[79,80], regeneration and synaptic plasticity during learning and memory[78,81]. As the transcriptome and translatome are dynamically regulated over time and upon activity[69], it is possible that the repertoire of localized mRNAs and their translation status changes across different subcellular scales in the intact circuitry of the brain.

Given the increased sensitivity of RNA detection in tissue, it is now possible to investigate whether principles of mRNA localization and local translation in cultured cells and simpler organisms apply to mammalian tissues. The further development of in situ transcriptomics and imaging technologies should enable the discovery of nanoscale structures and RNA localization patterns in healthy and diseased tissues across different organs.

## Localized cell biology

Key examples of mRNA localization and protein synthesis have been observed in different cell types (such as neurons and fibroblasts) and in organelles (such as mitochondria, the ER and centrosomes).

### Localization in different cell types

The subcellular control of gene expression is often restricted to discrete sites within polarized cells, including in neurons during synaptic connectivity and in fibroblasts during cell migration, owing to the asymmetrical distribution of mRNAs.

*Localization in neurons.* In neurons, the long-distance transport of mRNAs to dendrites and axons allows local translation in response to external signals, which is required for proper neuronal wiring during development and in processes such as learning and memory (reviewed in REFS[25,82,83]). The mRNAs encoding β-actin, ARC, CaMKIIα and BDNF, along with several others, are localized to dendrites and are responsible for the structural and functional remodelling of spines. The delivery of mRNAs to distal dendrites and to axon terminals, which are hundreds of microns away from the cell body, poses a notable challenge. Therefore, the packaging mRNAs into messenger ribonucleoprotein (mRNP) or granules (complexes of RNAs, RBPs, and the motor proteins dynein and kinesin) makes them 'transport ready' for movement along microtubules[84–86]. Although most studies indicate that mRNAs are translationally repressed during transport[87], emerging work using SunTag-based reporters indicate that translation may occur during transit[88] and/or in association with moving organelles[89] (FIG. 2). The accessibility of mRNAs

to ribosomes during transport is not well understood, and it is possible that the translational state of different mRNPs during transport may be regulated differently. How neurons achieve the precise and timed localization of each dendritic mRNA to specific synapses for local translation is still unclear (reviewed in REF.[25]). One idea is the 'RNA signature' hypothesis, indicating that each mRNA has a unique signature of regulatory elements that determines its transport, localization and translational control[90]. This RNA-centric view of how differential sorting is achieved and how the stability of these mRNAs determines transport and translation dynamics needs further investigation.

mRNA encoding β-actin (*ACTB* mRNA) is the most well-characterized, localized mRNA in neurons. In dendrites, at a given time, only 10–20% of *ACTB* granules move bi-directionally, and the majority of the remaining granules are stationary[91,92]. Similar patterns of movement have been observed for other dendritically localized mRNAs, indicative of a 'sushi-belt' model, whereby mRNAs patrol multiple spines in a conveyor belt waiting for cues to be captured at a specific spine[86] (FIG. 2a). Such cues include local synaptic activity, which can be mimicked in cell culture by uncaging the neurotransmitter glutamate on a subset of spines. This activation leads to the localization of *ACTB* mRNA to the base of these spines (~40% efficiency), possibly unmasking these granules for translation[87,91]. Although this model explains how *ACTB* mRNAs are locally enriched near stimulated spines, it is unclear whether this mechanism is conserved for other mRNAs. In particular, the validity of the model for low copy number and short-lived mRNAs, such as *ARC*, needs investigation. Once localized, mRNAs are usually translated in bursts of ~17 min, as observed using reporters containing the 3′ untranslated region (3′UTR) of β-actin[93], followed by translational shutdown. Newly synthesized actin proteins are proposed to participate in structural changes in the spines[91] (FIG. 2a). Again, the translation pattern for other dendritically localized mRNAs may vary, depending on the functions of their respective proteins in the spines.

Axons can grow up to a metre long and navigate across multiple lengths via growth cones during development to reach their postsynaptic targets. Growth cones require rapid protein synthesis to sense and undergo structural remodelling in response to extracellular cues, which is achieved by the presence of a readily translatable pool of mRNAs as seen in mammalian and *X. laevis* RGC neurons[69,72]. Indeed, localized *Actb* mRNA results in newly synthesized actin that enables growth-cone turning and synaptogenesis[70,94] (FIG. 2b). Similar to dendrites, 14% of *actb* mRNA molecules in *X. laevis* axons were actively moving, and a bias in the anterograde direction resulted in growth cone localization[94]. Besides actin, specific ribosomal proteins and mTOR may be locally synthesized in axons, playing roles in axon branching of the developing brain[95] and axonal regeneration after injury[73]. 3′UTR *cis*-regulatory elements that allow dendrite trafficking are also critical for the localization of mRNA in axons and growth cones, although consensus sequences that target mRNAs specifically to axons have not been identified. mRNAs may also hitchhike on
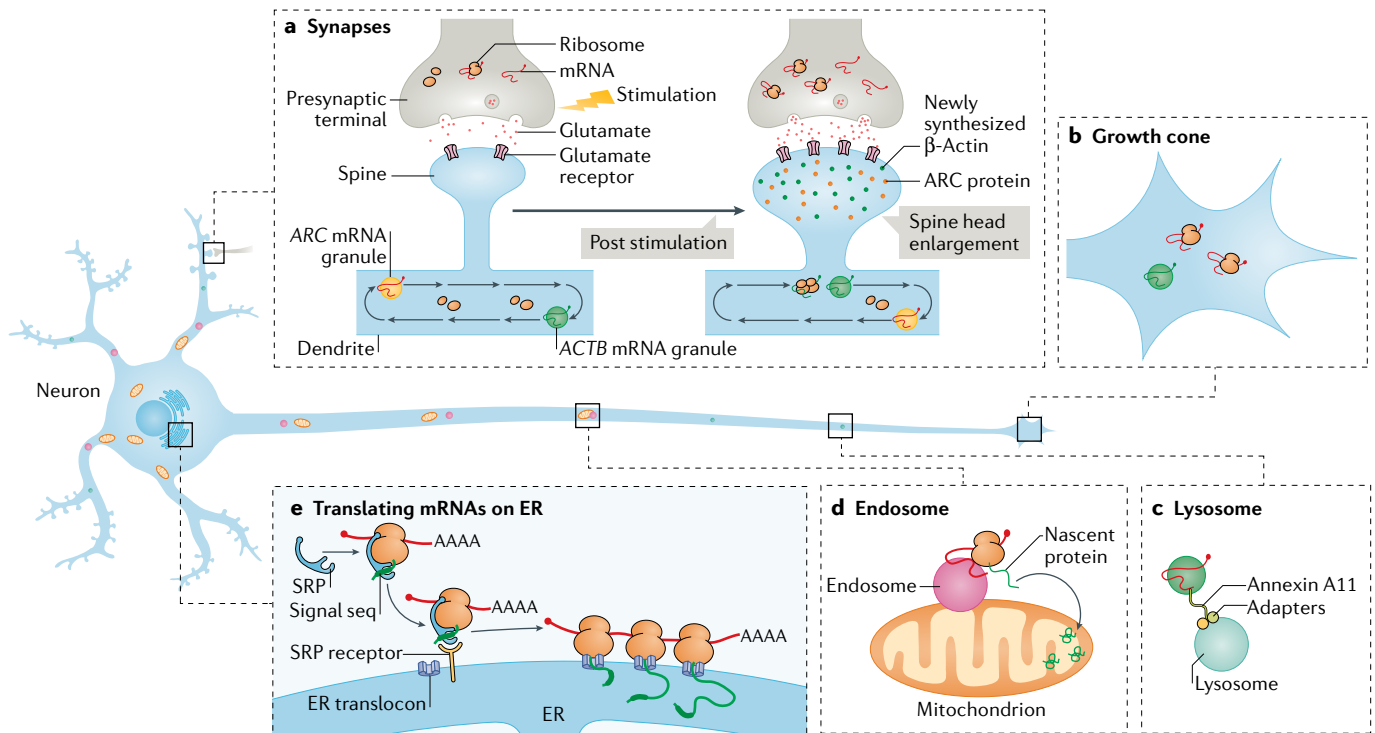
Fig. 2 | **Subcellular mRNA localization and local translation in neurons.** In neurons, mRNA localization and translation occur in processes (dendrites and axons). **a** | Neuronal transport granules, such as those containing *ACTB* and *ARC* mRNA, are trafficked along microtubules in dendrites like a conveyor belt patrolling multiple spines. The activation of specific synapses by stimulating the presynaptic terminal or by direct stimulation of postsynaptic spines using glutamate uncaging increases the binding of glutamate to the glutamate receptors. Synaptic stimulation leads to the capture of the moving mRNAs to the base of the stimulated spine, resulting in the localization and translation of mRNAs (for example *ACTB* mRNA). The newly synthesized proteins (green and orange dots) participate in enlarging the spine head and strengthening the synapse. Many dendritic mRNAs are localized following activity but it is unknown whether they all move and localize with similar kinetics. mRNA localization and local translation is also observed in the presynaptic compartment in response to stimulation. **b** | mRNAs such as *ACTB* mRNA are trafficked along axons to localize and translate in growth cones; this localization has critical roles in development and synaptogenesis. **c** | Long-distance mRNA transport in axons may also occur via the hitchhiking of mRNAs on lysosomes. The tethering of mRNAs to the lysosomal membrane occurs via proteins such as Annexin A11. **d** | Endosomes are often closely associated with the mitochondria and may behave as translation platforms for axonal mRNAs such as those encoding Lamin-B2 and VDAC2. The newly synthesized proteins are imported into and contribute to the function of mitochondria. **e** | mRNAs encoding secretory and membrane proteins are proposed to localize and translate using ribosomes on the endoplasmic reticulum (ER). Translation begins in the cytosol and the ER signal sequence on the nascent peptide gets bound by the signal recognition particle (SRP), which in turn binds to the SRP receptor on the ER membrane. Translation, often engaging polysomes, is resumed on the ER membrane and the nascent protein remains within the ER lumen, where it undergoes further processing.

organelles such as lysosomes and endosomes to achieve long-distance axonal transport[89,96] (FIG. 2c). However, although this presents an efficient and economical choice for the neuron, the specificity of mRNA localization is difficult to explain with this model.

Neurons must also localize the translation machinery. Although the presence of polyribosomes in dendritic shafts and at the base of spines is well documented[76,77], the paucity of these structures in presynaptic terminals[97,98] led to uncertainties about the translation state of localized mRNAs in mature axons. A recent study using polysome profiling followed by sequencing demonstrated that notable amounts of protein synthesis in the synaptic neuropil (a region enriched in both axons and dendrites) of the adult rodent brain are likely to occur on monosomes rather than on polysomes[99]; neuronal cell bodies exhibited more polysome-driven translation than distal processes. Similar monosome-driven translation has been observed in the cue-specific translation of receptor-specific mRNAs, such as *dcc* and

*nrp1*, in growth cones in RGC axons from *X. laevis*[100]. Monosomes potentially allow a diverse set of proteins to be produced from a limited pool of available ribosomes at synapses far away from the cell body[83,99].

The translation efficiency in different neuronal compartments can be increased by increasing local mRNA–ribosome interactions via organelles (FIG. 2d,e). For instance, endosomes may bring together the translation machinery and mitochondria, providing ATP for translation (see below)[89] (FIG. 2d). A notable number of synaptic proteins belong to the class of membrane proteins and secreted proteins and their translation on the ER is critical for proper folding[101] (FIG. 2e). Although mRNAs encoding these proteins have been detected in the processes, their capacity for local translation remains questionable. Finally, the translation machinery may also be delivered to distal extremities via exosomes, as shown for the delivery of ribosomes from Schwann cells to axons of the sciatic nerve[102] (reviewed in REF.[103]). Although neuronal activity leads to exosome release at

synaptic terminals[104,105], their contribution in effectively transferring genetic material and promoting translation requires further investigation.

The characterization of local translation in neurons has been extensively performed in primary cultures and often using overexpressed exogenous reporters. Although unravelling the molecular regulation of this process is useful, real-time imaging of mRNAs and their translation status in tissue are needed to identify how physiological activity affects the kinetics of their localization and translation. The tagging of endogenous genes with fluorescent proteins has been made possible in primary neurons with ORANGE, an optimized CRISPR–Cas9-based system[106]. So far, mRNAs encoding CaMKIIα, β-actin and PSD-95 have been tagged with the fluorescent protein Venus to study how chemically induced plasticity influences translation dynamics in real time[107]. It will be important to characterize the growing repertoire of localized mRNAs in neurons to elucidate which proteins need to be locally synthesized (for example, synaptic, cytoskeletal proteins and trophic peptides) for brain function.

*Localization at the leading edge of fibroblasts.* Cell migration requires actin polymerization to promote the formation of lamellipodia and filopodia at the cell leading edge. This was shown in cell types such as fibroblasts[108] and mesenchymal cells[109], and the discovery that mRNAs coding for β-actin localize in fibroblast protrusions suggested that local translation upholds specific protein networks in subcellular compartments[110]. In lamellipodia and filopodia, actin filaments are physically coupled to integrins, which are heterodimeric receptors that bind to the extracellular matrix[111]. Integrins cluster at focal adhesion complexes (FACs), where they provide the mechanical tension required for cell movement and act as a scaffold for signalling molecules and ribosomal proteins. Ribosomes accumulate at the leading edge[112], most likely through the interaction of the ribosomal kinase RACK1 with integrins[113]. Additionally, integrin–extracellular matrix binding is sufficient to recruit mRNAs[114] and translation initiation factors[115] to FACs to stimulate protein synthesis[116].

The most widely studied mRNA localized to protrusions is that encoding β-actin, which has bipartite zipcodes in its 3′UTR[110] (reviewed in REF.[117]) that are bound by zipcode-binding protein 1 (ZBP1; also known as IGF2BP1) with very high affinity[118–122]. The protein-interactome for *ActB* mRNA was mapped using a proximity-dependent biotin identification (BioID) assay in murine fibroblasts, which identified the RBP FUBP3 to be essential for the localization of *ActB* mRNA to the leading edge[122]. Besides the established roles of ZBP1 in translation regulation, it may also facilitate mRNA–motor binding[123] and active transport along actin and microtubules[124,125] (see below). In response to external cues or growth-factor stimulation, disassembly of the ZBP1–*ActB* mRNP occurs by Src-dependent phosphorylation of ZBP1 at the leading edge to allow the local translation of *ActB* mRNA[126]. The simultaneous imaging of fluorescently labelled 60S ribosomal subunits and single endogenous *ActB* mRNAs revealed that, in

protrusions, efficiently translated mRNAs are associated with a high number of ribosomes[127], namely polysomes. Interestingly, the accumulation of ribosomes next to FACs decreases mRNA diffusion speeds from $0.4\,\mu m^2/s$ to $0.1\,\mu m^2/s$, indicative of local translation[128]. Of note, inferring the translation status based on subtle changes in diffusion coefficients is often difficult, and further studies are required to establish a functional link between the local translation of *ACTB* mRNA at the FACs and cell migration.

A plethora of mRNAs besides *ACTB* mRNA are differentially distributed in polarized cells, but the extent to which their local translation maintains the front–back asymmetry necessary to promote cell migration is unclear. RNA-sequencing and pulse-SILAC have been combined to determine the relative translation rates (BOX 3) between protrusions and the cell body. The mRNAs encoding for actin cytoskeleton-associated proteins were not enriched at the protrusions despite being heavily translated there. In fact, the translation of mRNAs encoding for mitochondrial and ribosomal proteins was repressed at the protrusions, despite these mRNAs being locally enriched[129]. Given that the number of mRNAs does not always correspond to translation efficiency, translation rates will have to be determined at single-molecule resolution in living cells in order to fully understand the role of the localization of each mRNA.

## Localization at different organelles

Organelles mediate compartmentalization and the regulation of many intracellular processes. Protein targeting to organelles occurs via peptide sequence-based targeting to a specific organelle or via mRNA localization and in situ translation. With increased imaging resolution and proximity labelling assays (BOX 3), how mRNAs are regulated at the subcellular scale on different organelles as well as the potential implications on organelle biogenesis and maintenance have been studied.

*Outer mitochondrial membrane.* Mitochondria are essential for ATP synthesis through oxidative phosphorylation, and their activity impacts all aspects of cell physiology. Mitochondria distribution within cells is dynamic, and continuous reshaping of the mitochondrial network supports the local energy demands. For instance, in neurons, mitochondria in dendrites supply the energy required for local translation and synaptic plasticity[130]. Nuclear transcripts encoding mitochondrial proteins, including those that encode mitochondrial inner membrane proteins, are enriched near the mitochondria[52,131,132]. Using the MS2–MCP system in *S. cerevisiae*, up to 24 mRNAs were dynamically visualized in the proximity of mitochondria and in response to changing environments[51,133].

The 5′UTR and 3′UTR sequences of mRNA control its localization near mitochondria. In yeast, the 3′UTR contains a *cis*-regulatory element bound by RBP Puf3, which directs the mRNA to mitochondria[51,134]. In *D. melanogaster* and mammalian cells, the 5′UTR interacts with the outer mitochondrial membrane (OMM) kinase PINK1. Interestingly, in mammalian cells, the translation of nuclear-encoded mRNAs coding for mitochondrial

proteins is regulated by mTOR[135]. mTOR phosphorylates a family of inhibitory proteins called 4E-binding proteins (4E-BPs). Phosphorylated 4E-BPs are released from the cap-binding protein eIF4E to promote the assembly of the eIF4F complex, the composition of which is dictated by specific features of the 5′UTRs of nuclear-encoded mitochondrial mRNAs[136]. Thus, the 5′UTR of nuclear-encoded mitochondrial mRNAs may coordinate their localization and translation.

Although the majority of mitochondrial proteins are synthesized by cytoplasmic polysomes and imported via peptide-dependent targeting[137–139], proximity-specific ribosome profiling (BOX 3) revealed the translation of certain nuclear-encoded mitochondrial mRNAs near the OMM in yeast[140] and higher eukaryotes[141]. However, these studies lacked the resolution to elucidate whether ribosomes are tethered to OMM for co-translational import or if they are present in close proximity to the membrane. High-resolution electron cryo-tomography resolved this conundrum by revealing ribosomes on the surface of OMM with the peptidyl exit tunnel oriented to favour the import of elongating polypeptides into the mitochondria. Furthermore, ribosomes appeared to be tethered to the import pore translocase of the outer membrane complex by nascent polypeptides[142]. Translating ribosomes and mitochondria appear to be associated through an interaction between the ribosome-associated nascent chain-associated complex and the OMM protein (Om14 in yeast) to support co-translational mitochondria import or, as described in *D. melanogaster* and mammalian cells, through an interaction between the mitochondria-targeting sequence in the nascent peptide and the receptor Tom20 (REF.[143]).

Single-molecule imaging using the SunTag system (BOX 3) revealed that mitochondrial protein synthesis is also facilitated by the physical association of mitochondria with late endosomes[89]. This interaction occurs in *X. laevis* RGC axons, in which late endosomes contact mitochondria for over 2 min and serve as translation sites for mRNAs encoding proteins such as Lamin-B2 and VDAC2. These newly synthesized proteins are likely imported into mitochondria to perform structural functions. Furthermore, the global perturbation of late endosomes by the disruption of Rab7a function impairs local mRNA translation but not mRNA localization. Interestingly, the disruption of the axonal translation of mitochondria mRNAs, as occurs in Charcot–Marie–Tooth type 2B neuropathy, impairs mitochondrial integrity and axonal activity[89].

Further work is needed to understand whether the spatiotemporal regulation of local translation at or near the OMM affects mitochondria biogenesis and physiology.

*Endoplasmic reticulum.* Electron microscopy studies have identified two major populations of ribosomes in cells: ER-bound ribosomes and those freely diffusing in the cytoplasm. Although cytoplasmic ribosomes are abundant in all cell types[144], ER-bound ribosomes are enriched in secretory cells, where they preferentially translate mRNAs encoding secreted and integral membrane proteins. The translation of ER-bound mRNAs

begins in the cytoplasm. Once a nascent polypeptide containing a hydrophobic domain (that is, a signal sequence) emerges from the ribosome, it is recognized by the SRP complex. SRP binding halts translation until the mRNA is relocated to the ER[145] (FIG. 2e). This dynamic process has been visualized by imaging the nascent polypeptide on translating mRNA at single-molecule resolution[93]. Briefly, the N-terminal domain of the membrane protein cytochrome p450, which anchors the transmembrane domain into the ER membrane[146], was fused to a SunTag peptide array, and the encoding mRNA was simultaneously imaged with the MS2 system. Real-time imaging of this reporter mRNA confirmed that translation starts in the cytoplasm and elongation occurs on the ER[93]. Thus, mRNA localization to the ER is translation-dependent and SRP peptide sequence-dependent and differs from 'zipcode'-dependent mRNA localization.

Several studies have challenged the canonical models predicting that the translation of cytoplasmic mRNAs is excluded from ER membranes. Cell fractionation and genome-wide approaches have identified cytoplasmic mRNAs and translation initiation factors in proximity of the ER, suggesting that de novo translation of cytosolic mRNAs can occur on the ER membrane[147]; however, the physiological relevance is unclear. *GAPDH* mRNA is a classic example of ER-associated cytosolic mRNA[148–150]. However, given the abundance of this housekeeping transcript and the resolution of the methodologies used, these findings need further validation. For improved spatial resolution, smFISH (Supplementary Box 1) was employed to detect *GAPDH* and other cytosolic mRNAs associated with the ER membrane upon a mild digitonin extraction, which removes soluble cytoplasmic molecules while preserving ER integrity[151]. Imaging with the translation reporter 'translating RNA imaging by coat protein knock-off' (TRICK)[152] (BOX 3) in living cells revealed that a small fraction of cytosolic mRNAs was indeed translated on the ER. Interestingly, the number of ribosomes in the ER extract was higher compared to the cytosolic counterpart, suggesting that the translation of cytoplasmic mRNAs may be more efficient at the ER than in the cytosol[151].

Compartmentalizing mRNAs to the ER may play a critical role in maintaining the translation of specific mRNAs under stressful conditions. Viral infection and other cytotoxic stresses suppress cytoplasmic cap-dependent translation while ER membrane-bound mRNAs are able to partially escape this silencing[153,154].

*Centrosomes.* Centrosomes — membraneless organelles composed of two centrioles surrounded by the pericentriolar material[155] — are the microtubule-organizing centre of the cell and participate in chromosome segregation and cell division. The asymmetric localization to and local translation of mRNAs at the centrosome may contribute to asymmetric cell division, leading to embryonic pattering and the selective inheritance of specific transcripts[21–23,156,157].

Cell cycle-dependent mRNA localization to centrosomes can be translation dependent or translation independent. The translation-dependent transport of mRNAs is observed in zebrafish and mammalian

cells, where mRNAs are co-translationally delivered to centrosomes through a microtubule-dependent and dynein-dependent process[22,156–158]. Polysomes translating *PCNT* mRNA, encoding a core component of the pericentriolar material, are attached to the dynein motor complex through the LIC1 domain located in the N-terminal of the *PCNT* nascent peptide both in zebrafish and mammalian cells[22]. In mammalian cells, the translation-dependent localization of *PCNT* mRNA and of the mRNA encoding the microtubule minus-end regulator ASPM to the centrioles enables protein localization within 30 min. ASPM and the microtubule-binding protein NUMA1 were tagged by combining the SunTag and MS2 systems to label the nascent peptides and the mRNA, respectively (BOXES 2,3), further demonstrating that mRNA localization depends on the nascent peptide connecting polysomes to motors. Both mRNAs, when translationally active, were directly and actively transported to centrosomes[156,158]. Finally, in quiescent, immortalized human retinal pigment epithelial cells, *NIN* mRNAs, encoding a core component of centrosomes, localize at the centriole basal body found at the base of cilia[157]. *NIN* mRNA localization is both translation dependent and exon junction complex dependent[157], suggesting a tight connection between the different steps of mRNA processing.

The translation-independent, cell cycle-dependent localization of mRNA to centrosomes occurs via the interdependent localization of two mRNAs to the centrosome. In *D. melanogaster*, a pair of antisense mRNAs, *Ik2* (encoding IκB kinase like 2) and *Cen* (encoding centrocortin), share complementary sequences in their 3′UTRs, leading to base-pairing and co-transport[23]. Both mRNAs localize to the centrosome but the localization of *Ik2* depends on that of the *Cen* transcript. The simultaneous localization of *Cen* mRNA and nascent peptides suggests that *Cen* mRNA is locally translated at the centrosome. Interestingly, the orthogonal analysis of APEX-seq data indicates that antisense mRNA pairs tend to co-localize in specific subcellular compartments[23], suggesting a novel mechanism of coordinated mRNA localization.

## Mechanisms of mRNA localization

Three major mechanisms control the localization of mRNAs to subcellular compartments: directed transport, protection from mRNA degradation, and passive diffusion and local entrapment.

### Localization by active mRNA transport

Active transport is the most common mode of mRNA localization reported in all eukaryotic cells. Motor-driven transport in cells and organisms occurs on actin filaments or microtubules (FIG. 3a–d). Here, mRNAs are targeted through the binding of RBPs to *cis*-regulatory elements, which are characterized by unique secondary structures and dubbed 'zipcodes'. These *cis*-elements are found in 3′UTRs and 5′UTRs as well as in coding regions (reviewed in REFS[19,33,159]) and can vary in length and in the diversity of RBPs recognizing them. The recognition is based on the primary sequence (for example, a 54-nulceotide sequence with bipartite motifs in
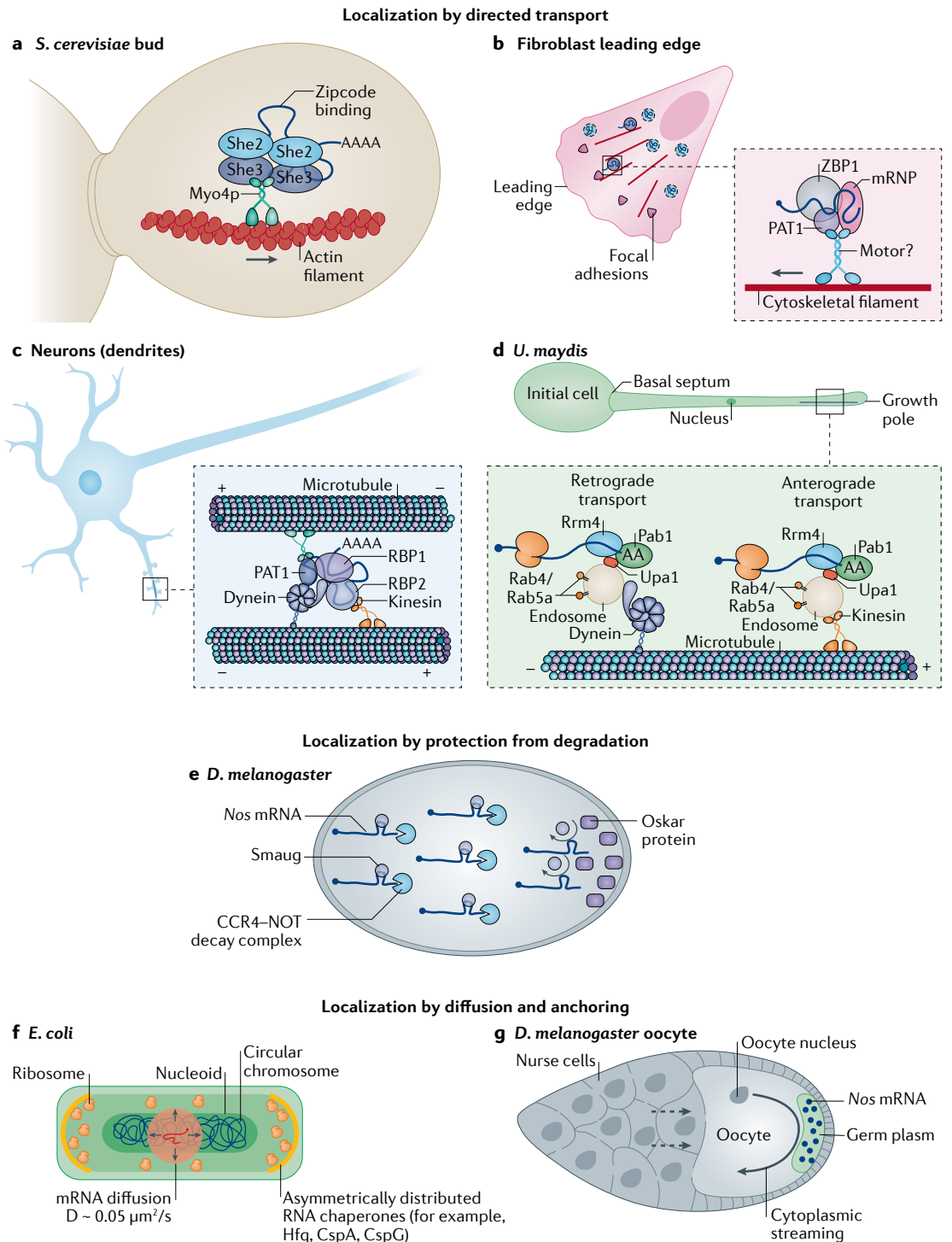
chicken and mouse *Actb* mRNA is specifically bound by ZBP1 (REF.[117])) and also on the basis of complex secondary structures (for example, the zipcode structure of *S. cerevisiae ASH1* mRNA[160], the helical structure of *D. melanogaster bcd* mRNAs, and G-quadruplexes in neuronal mRNAs such as those encoding CaMKIIα and PSD95)[161]. Across different cell types and organisms, mRNAs are moved by myosin, kinesin and dynein motors. How different RBPs recruit motor proteins to form a localization-competent transport granule is unclear and complicated by the fact that a single mRNA may be bound by multiple RBPs with unknown roles in transport regulation. Evidence of an RBP directly binding to motor proteins and bridging the interaction with the mRNA comes from yeast, where She3 binds Myo4 to move mRNAs along actin, although RBPs can also indirectly engage motors via adaptor proteins. Indeed, the APP tail 1 (PAT1) protein is a direct adapter between ZBP1 and the kinesin 1 motor complex, facilitating the neuronal activity-induced transport of *ActB* mRNA to dendrites[162].

*Transport on the actin cytoskeleton.* In yeast, bud-localized *ASH1* mRNA has four zipcodes, distributed across the coding region and the 3′UTR, that are recognized co-transcriptionally by the RBP She2 and its partner Loc1 (REFS[163–167]). The synergistic binding of She2 dimers to the *ASH1* mRNA induces a conformational switch that promotes high-affinity mRNA–She2 binding[160]. Loc1 is dissociated from the mRNP before export into the cytoplasm[168], and the She2–mRNA complex is intercepted by She3, which is constitutively bound to the type V myosin motor Myo4 (REFS[160,165–167,169]). This interaction promotes the formation of a translocation-competent mRNP that is actively transported to the bud on actin filaments (FIG. 3a). Besides *ASH1* mRNA, tens of mRNAs, including *CLB2*, *TCB2*, *TCB3* and *IST2* (REFS[35,170]), interact with the She2–She3 complex and are actively transported along actin filaments. Further work is required to characterize how their transport and local translation is coordinated. In mammalian cells, the transport of *Actb* mRNAs to the leading edge of migrating fibroblasts occurs on both actin and microtubules, with ZBP1 playing roles in the transport via its interaction with motor proteins[123,124,171] (FIG. 3b). Interestingly, interactions between actin and myosin may contribute to transport efficiency[124] but whether ZBP1 results in increased myosin motor engagement needs further study.

*Transport on microtubules.* In mammalian cells, motor protein-based active transport is best illustrated by the localization of mRNAs to distal dendrites and axon terminals in neurons via the movement of transport granules (comprising RNAs with RBPs and dynein and/or kinesin) along microtubule tracks[84–86] (FIG. 3c). In other cell types, such as in fibroblasts and epithelial cells, active transport localizes a small percentage of mRNAs, and corralled diffusion is often sufficient to move mRNAs[128] (FIG. 3b). *ACTB* mRNA is the best-characterized neuronal transport granule and moves processively at speeds of 0.5–2 μm/sec in both dendrites and axons[91,92]. Interestingly, these

APEX-seq
Proximity labelling of RNA with biotin using the peroxidase enzyme APEX (or APEX2) followed by sequencing.

**Localization by directed transport**

a  *S. cerevisiae* bud

Zipcode binding
She2 / She2
She3 / She3
AAAA
Myo4p
Actin filament

b  Fibroblast leading edge

Leading edge
Focal adhesions
ZBP1
mRNP
PAT1
Motor?
Cytoskeletal filament

c  Neurons (dendrites)

Microtubule
+  −
AAAA
RBP1
PAT1
RBP2
Dynein
Kinesin
−  +

d  *U. maydis*

Initial cell
Basal septum
Nucleus
Growth pole

Retrograde transport
Rrm4
Pab1
AA
Rab4/Rab5a
Upa1
Endosome
Dynein

Anterograde transport
Rrm4
Pab1
AA
Rab4/Rab5a
Upa1
Endosome
Kinesin
Microtubule
−  +

**Localization by protection from degradation**

e  *D. melanogaster*

*Nos* mRNA
Smaug
CCR4–NOT decay complex
Oskar protein

**Localization by diffusion and anchoring**

f  *E. coli*

Ribosome
Nucleoid
Circular chromosome
mRNA diffusion D ~ 0.05 μm²/s
Asymmetrically distributed RNA chaperones (for example, Hfq, CspA, CspG)

g  *D. melanogaster* oocyte

Nurse cells
Oocyte nucleus
*Nos* mRNA
Germ plasm
Oocyte
Cytoplasmic streaming

speeds are similar for other microtubule-dependent, dendritically localized mRNAs, such as *ARC*[172], and for exogenous reporters containing 3′UTRs with localization elements[173,174]. Mostly, dendritic mRNAs exhibit oscillatory movement, switching between anterograde and retrograde directions. This bi-directionality has been attributed to the mixed orientation of the microtubules and a possible 'tug-of-war' between the dynein and the kinesin motors, which together determine the net movement direction[25,159]. One limitation of motor-driven transport is the energy cost for the cell, especially for long

axons. To circumvent this problem, mRNAs can hitch-hike on organelles such as lysosomes for long-distance transport in axons[96] (FIG. 2c). The tethering of mRNAs on actively moving lysosomes is facilitated by Annexin A11 via its intrinsic membrane binding and phase-separating domains. Similar hitchhiking on early and late endosomes has been observed for axonal mRNA transport[89].

This hitchhiking strategy also occurs in filamentous fungi, including in the corn plant pathogen *U. maydis*, in which a close link between mRNA transport and the endocytic pathway was demonstrated[56] (FIG. 3d).

◀ Fig. 3 | **Modes of mRNA transport and localization in cells and organisms. a** | Several mRNAs are localized to the bud of *Saccharomyces cerevisiae*. She2 dimerizes and binds these mRNAs via their zipcodes, before binding She3, which bridges the interaction of the complex with the type V myosin motor Myo4. The ribonucleoparticles are actively transported along actin filaments. **b** | In mammalian fibroblasts, mRNAs such as those encoding β-actin are localized to the leading edge by RNA binding proteins (RBPs) such as ZBP1, which binds to the zipcode on the 3′ untranslated region (3′UTR) of the mRNAs to form messenger ribonucleoproteins (mRNPs) that associate with unidentified motors. PAT1 acts as a direct adapter between ZBP1 and the motor. This represents a small percentage of mRNA movement as the majority of mRNAs undergo corralled cytoplasmic diffusion (indicated by the dashed boundaries). **c** | Localization to distal spines is achieved by packaging mRNAs involved in synaptic remodelling into transport granules composed of RBPs, the minus-end-directed motor dynein and the plus-end-directed motor kinesin. Due to the mixed polarity of microtubules in dendrites and the presence of both motors, these granules move bi-directionally (that is, in anterograde and retrograde motion). The net movement is proposed to occur by a 'tug-of-war' between the motors determined by their stoichiometry. **d** | In *Ustilago maydis*, cells switch from yeast to filamentous growth to promote plant invasion. To sustain asymmetric growth, protein, ribosomes and mRNA are transported to the growth pole. mRNAs are bound to endosomes via the endosome membrane-binding protein Upa1, which mediates the interaction with the RBPs Rrm4 and Pab1. Bi-directional mRNA transport on microtubules occurs via kinesins (anterograde motion) or dyneins (retrograde motion). **e** | In *Drosophila melanogaster* embryos, *Nos* mRNAs are bound by the RBP Smaug, which recruits the CCR4–NOT complex to initiate mRNA decay. At the posterior pole, however, *Nos* mRNAs are protected from degradation by Oskar proteins, which displace Smaug to increase local concentrations of *Nos* mRNAs. **f** | In *Escherichia coli*, mRNAs localize to ribosome-rich poles or to the membrane by random diffusion at speeds of 0.05 μm²/s, aided by the chaperone proteins that anchor the mRNAs. **g** | During *D. melanogaster* oogenesis, several hundreds of mRNAs are deposited to the oocyte by nurse cells (dashed arrows). mRNAs such as *nos* are localized to the posterior pole of the oocyte by cytoplasmic streaming and entrapped in the germ plasm in an actin-dependent manner.

Endosomes, which are membrane-bound vesicles formed during endocytosis, are important for membrane and lipid trafficking and for transporting cargos such as membrane proteins, cell debris, bacteria and viruses as well as mRNAs[175]. Work in *U. maydis* showed that the *CDC3* mRNA and the encoded septin protein, a key regulator of unipolar growth, are co-transported on the same endosomes[57,176]. Ribosomal proteins are also co-transported, suggesting that translation occurs during trafficking[176]. The RBP Rrm4 is essential for shuttling both the mRNAs and ribosomal proteins, further corroborating the hypothesis of 'in-motion' mRNA translation[176,177]. The mRNA and the RPB Rrm4 as well as the poly-A binding protein Pab1 interact with the endosome via the membrane-bound protein Upa1 (REF.[59]). This complex is transported bi-directionally on microtubules by the plus-end-directed kinesin Kin3 or by the minus-end-directed dyneins Dyn1 and Dyn2 (REFS[178,179]). As mRNA localization coupled to vesicle transport (endosomes or lysosomes) also occurs in neuron cells[89,96], this active-transport pathway might be highly conserved.

Therefore, future identification of the RBPs and the motors associated with transport granules from different cellular compartments will provide insights into the reorganization of granules (see below). Although motors are bound to mRNAs until they reach their final destination, it remains unclear whether motor proteins are exchanged during granule movement, whether motors are disengaged from mRNAs upon localization and what molecules halt mRNA movement.

## Protection from mRNA degradation

In the *D. melanogaster* embryo, cell-fate specification is achieved by the precise distribution and translation control of mRNAs encoding patterning factors. Cytoplasmic *Nos* mRNA is targeted by the RBP Smaug (via the Smaug recognition element located in the 3′UTR), which inhibits *Nos* mRNA translation and recruits the CCR4–NOT complex to trigger *Nos* mRNA decay in the cytoplasm[180,181]. Later in development, in the germ plasm at the posterior pole, Oskar displaces Smaug from the *Nos* mRNAs, thus protecting the mRNA from degradation and relieving translation inhibition[181] (FIG. 3e). The *Hsp83* mRNA was protected from degradation by a similar mechanism although the Smaug recognition element is located in the coding sequence of *Hsp83* mRNA[182–184]. Around 300 mRNAs are associated with Smaug in *D. melanogaster*[182]; however, further investigations are required to elucidate if they are protected from degradation by Smaug and if this regulates their localization.

## Passive mRNA diffusion and anchoring

In tiny organisms like bacteria, biomolecules diffuse rapidly within the 1–2 μm long cell[26,27]. mRNAs typically move with a diffusion coefficient of 0.05 μm²/s. These parameters suggest that the localization to ribosome-rich poles or to the membrane could occur within seconds of mRNA exiting the nucleoid, a timeframe notably shorter than the half-life of bacterial mRNA (~5 min)[185]. Consistent with these findings, no active mRNA transport mechanism has been observed in bacteria to date, even though the directed transport of proteins has been previously described[186]. Furthermore, the asymmetric distribution of RNA chaperones (for example, Hfq, CspA and CspG) could partake in the anchoring and localization of mRNA[187] (FIG. 3f); however, additional studies are required to elucidate the mechanisms governing these events.

During *D. melanogaster* oogenesis, nurse cells contract, squeezing their cytoplasm and depositing hundreds of mRNAs into the oocyte, including *Nos* mRNA[10]. *Nos* mRNA diffuses towards the posterior pole owing to the cytoplasmic streaming that microtubules generate for transport[20,24]. Once at the posterior pole, *Nos* mRNAs are entrapped in the germ plasm in an actin-dependent manner (FIG. 3g). *Nos* mRNA localization and localized translation are essential for the anterior–posterior body axis patterning in *D. melanogaster*[62,188].

## Regulation of local translation

Although it is clear that the subcellular localization of mRNA regulates gene expression, the mechanisms regulating localized mRNA translation are only just emerging. The development of methods that can simultaneously measure mRNA localization and localized translation in fixed and living cells (BOX 3) are starting to unravel how translation factors and ribosomes are locally regulated to modulate protein synthesis, revealing that a major control step occurs during translation initiation.

### Inhibition of translation by RBPs

A number of RBPs that are involved in mRNA transport also play roles in translation repression, thereby coupling the regulation of both processes[189]. For example,

ZBP1-bound *ACTB* mRNA is packaged into transport granules, which are translationally silent during trafficking in dendrites and fibroblasts. Only upon localization to activated spines in neurons and to the leading edge in fibroblasts ZBP1 is phosphorylated, which unmasks mRNAs for translation[117,126]. Similarly FMRP, which is widely expressed in human and mouse, plays a role in transporting mRNAs in translationally repressed granules[190]. In *X. laevis*, RBPs, such as heterogeneous nuclear RNPs, bind to both ribosomes and mRNAs coding for guidance receptors in the growth cones, thereby keeping the mRNAs in a translationally repressed state under basal conditions. Upon cue stimulation, the binding of the cognate ligand to the specific receptor (DCC or Nrp1) causes the RBP to dissociate and triggers the translation of the specific receptor mRNA[100].

All eukaryotic mRNAs possess a 5′-end cap structure, and cap-dependent translation is a multistep and highly regulated process orchestrated by a network of translation initiation factors. At initiation, the cap-binding protein eIF4E recruits eIF4G and the helicase eIF4A to the mRNA and assembles the eIF4F complex[191]. The eIF4F complex in turn contacts elements of the 48S translational initiation complex[192] (FIG. 4a), and polypeptide synthesis starts when small and large ribosomal subunits join the mRNA at the start codon.

Cytoplasmic polyadenylation element binding proteins (CPEBs) are a family of well-characterized RBPs that inhibit pre-initiation complex formation. CPEB1 binds near the 3′UTR of mRNA and recruits a set of 4E-BPs, such as Maskin in oocytes[193], Cup in *D. melanogaster*[194] and Neuroguidin in neurons[195]. By binding to eIF4E, these proteins block the interaction between eIF4G and eIF4E, thereby interfering with translation initiation (FIG. 4b). Similarly, the binding of eIF4E to FMRP and the cytoplasmic FMRP-interacting protein 1 (CYFIP1) inhibits eIEF4F complex formation in neurons[196], although the role of this interaction in inhibiting translation is debatable as most studies point to a more direct role of FMRP in inhibiting translation elongation[197,198]. RBPs such as ZBP1 negatively impact the assembly of the 80S complex by impairing the joining
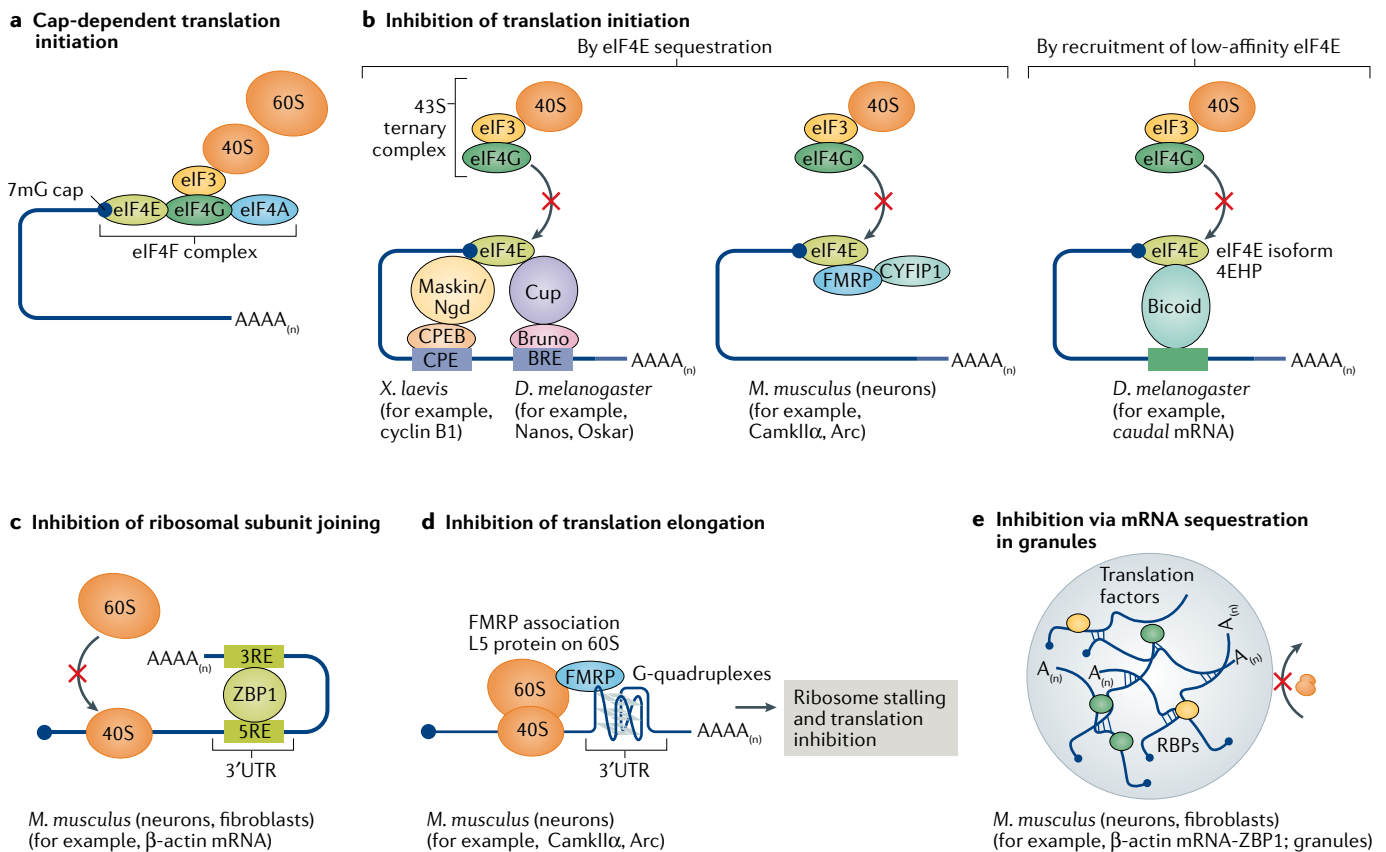


Fig. 4 | **Regulation of translation by RNA binding proteins. a** | Eukaryotic cap-dependent translation initiation occurs when the 40S ribosomal subunit binds to the 7mG-containing cap at the 5′ end of the mRNAs via an interaction involving eIF3 and the eIF4F complex of initiation factors eIF4A–eIF4G–eIF4E. **b** | Translation initiation is prevented when eIF4E (bound to the cap) is sequestered by 4E-binding proteins (4E-BPs) such as Maskin (in *Xenopus laevis*) and Cup (*Drosophila melanogaster*). These 4E-BPs are tethered to the mRNA by RNA binding proteins (RBPs) such as cytoplasmic polyadenylation element binding protein (CPEB) and Bruno, which bind to the *cis*-regulatory elements CPE and BPE, respectively, in the 3′ untranslated region (3′UTR) of the mRNA. In mammalian cells, RBPs such as FMRP and cytoplasmic FMRP-interacting protein 1 (CYFIP1) may directly interact with eIF4E and prevent it from binding to the preinitiation complex. Also, in *D. melanogaster*, RBPs such as Bicoid bound to the mRNA recruit an isoform of eIF4E known as 4EHP, which has a low affinity for eIF4G and is therefore unable to initiate translation. **c** | Some RBPs, such as ZBP1, do not impact the initiation of translation but prevent the 60S ribosomal subunit from joining the 40S subunit to assemble the 80S complex. **d** | RBPs may also stall elongating ribosomes as seen when FMRP binding to the L5 protein on the 60S subunit halts translation. **e** | Several RBPs, via protein–protein interactions, may sequester multiple mRNAs into transport granules or stress granules, which are believed to be mostly translationally silent.

of the large and small ribosomal subunits[126], a mechanism that is predominant in the perinuclear region of fibroblasts and in neuronal transport granules[120] (FIG. 4c). Some RBPs, for example FMRP, may operate at several steps; besides negatively affecting initiation, FMRP blocks elongation by binding to the L5 protein on the 80S ribosome, precluding the binding of tRNAs and translation elongation factors[197] (FIG. 4d). Finally, several RBPs can cluster mRNAs into higher order granules via protein–protein interactions (see below) such as in stress granules and transport granules, in which ribosomes and the other components of the translation machinery have limited accessibility (FIG. 4e).

### Promoting translation in 'translation factories'

How translation initiation is regulated upon mRNA localization is not fully understood, and this question is further complicated by the existence of several translation factor homologues as well as of ribosomes with heterogeneous composition[199]. One concept is that 'translation factories', that is, molecular assemblies promoting de novo translation, exist in the cytoplasm[156,158]. It has been proposed that eIF4F variants cluster together in response to extracellular stimuli[200], although it is not clear which fraction of the variants are employed in local factories. A recent translation biosensor designed to compare cap-dependent and IRES translation efficiency at single-molecule resolution demonstrated that translating mRNAs are in close proximity of each other while excluding non-translating mRNAs[201]. Additionally, mRNAs undergoing cap-independent translation localize more closely to the nucleus than mRNAs translated in a cap-dependent manner[201]. In *X. laevis* growth cones, translation factories have been observed close to the membrane, and cue-specific stimulation promotes the synthesis of certain receptor-associated mRNAs, such as *ctnnb1* mRNA (encoding β-catenin), that act locally at those specific receptors[100]. This observation suggests that translation preferentially occurs in some subcellular regions, creating translation hotspots or foci for specific mRNAs[202]. The concept of translation in specific foci was initially proposed in neuronal dendrites[203] and subsequently identified in both shafts and in spines[204–206], axonal branch points[192] and, recently, in live axon terminals in the intact brain[72]. In non-neuronal cells, the accumulation of mRNAs in distinct cytoplasmic foci along with their encoded proteins (for example, BUB1, DYNC1H1, β-catenin (encoded by *CTNNB1*) and ASPM) has been observed to occur in a translation-dependent manner[156,158]. These foci, in which the translation machinery is localized either separately or along with the mRNAs, have been termed translation factories. Based on these studies, whether 'hotspots' and 'factories' can be used interchangeably to indicate the clustering of translating mRNAs needs to be elucidated.

Furthermore, in budding yeast, mRNAs coding for translation initiation, elongation and termination factors are packaged in 'translation factor mRNA granules'. These granules are localized to the bud in a She2–She3-dependent manner, that is, via the same complex that transports bud-localized mRNAs[53]. Similarly, in neurons, ribosomes may be co-trafficked with mRNAs in dendrites, and assembly of the ribosome subunits occurs once translation repression is relieved by synaptic activity[87]. It is well known that several translation initiation factors localize to and are stored in stress granules in response to harmful situations in eukaryotes[207,208]. Finally, translation initiation can be promoted indirectly through the control of ribosome availability. As discussed above, in the intestinal epithelium, the apical localization of mRNAs encoding ribosomal proteins leads to a local increase in ribosome concentration, boosting the translation of apically localized mRNAs[67] (FIG. 1e).

Creating translation hotspots and factories is an effective way for a cell to translate rapidly in response to cues and to allow these newly synthesized proteins to act locally without perturbing the protein homeostasis of the entire cell. Further work is required to elucidate the coordinated localization of mRNAs and the translation machinery. In eukaryotes, recent ribosomal profiling has provided a systematic analysis of the mechanisms controlling the co-translational assembly of multi-subunit protein complexes[209,210]. The development of imaging techniques that allow the simultaneous visualization of multiple translated mRNAs and regulatory factors will be critical to characterize the coordinated translation of multiple components in situ and to determine the composition and function of these translation factories or hotspots.
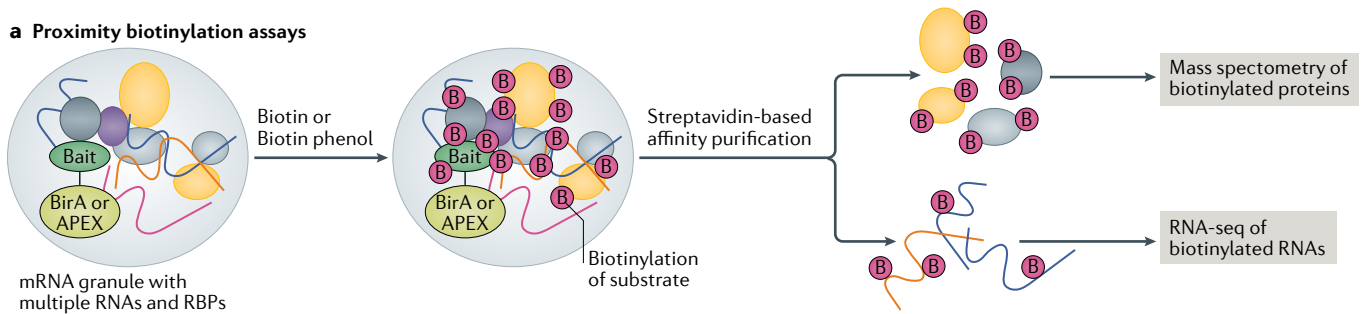
### RNA composition and fate in granules

Across different cell types and organisms, mRNPs are packaged into larger RNA granules. They come in different shapes and sizes and play roles in mRNA trafficking in neurons (transport granules) and in *D. melanogaster* (germ granules) as well as in storage and translational regulation in stress granules and P-bodies. Most granules are highly dynamic in nature; therefore, a unified model of granule assembly and disassembly is lacking owing to the technological challenges of accurately identifying the stoichiometry of all granule components at a given time. Multiplexed FISH and methods to map RNA–protein interactions using candidate-based approaches such as smFISH-IF[211] and global approaches such as APEX-seq[132,212] opened avenues for determining the complexity of granule composition (FIG. 5a,b, Supplementary Box 1). The term 'granule' has been loosely used to indicate all large assemblies of RNAs and proteins that promote selected mRNPs to come together and distinguish themselves as a granule.
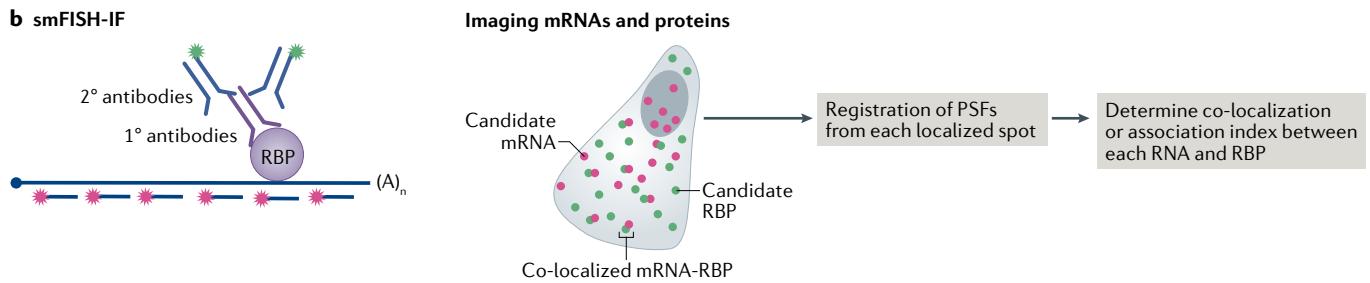
The classical view of granule formation relies on the binding of RBPs to the *cis*-regulatory elements in RNA. Mutations or deletions of the *cis*-elements or alterations in the secondary structure of the mRNA notably impact RBP binding and granule formation, leading to mRNA mislocalization in yeast[160], *D. melanogaster*[213] and neurons[214]. From a perspective of RBP-driven assembly, the same RBP may be shared between multiple mRNAs or interactions between multiple RBPs may coalesce many individual mRNPs into larger complexes and granules (see REF.[215] and FIG. 5c). Furthermore, by virtue of their intrinsically disordered regions, RBPs drive liquid–liquid phase separation (LLPS) and assemble

**Germ granules**
RNA-rich membraneless cytoplasmic granules found in the germline of organisms such as *X. laevis*, *D. melanogaster* and *D. rerio*.
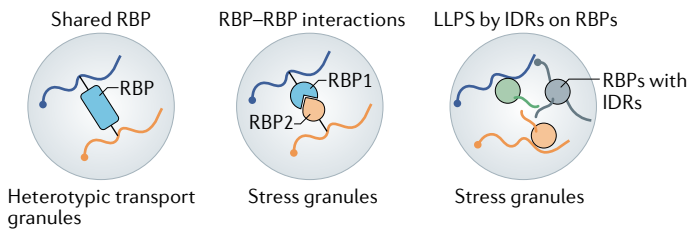
**a  Proximity biotinylation assays**

mRNA granule with multiple RNAs and RBPs

Biotin or Biotin phenol

Biotinylation of substrate

Streptavidin-based affinity purification

Mass spectometry of biotinylated proteins

RNA-seq of biotinylated RNAs

**b  smFISH–IF**

2° antibodies

1° antibodies

**Imaging mRNAs and proteins**

Candidate mRNA

Candidate RBP

Co-localized mRNA–RBP

Registration of PSFs from each localized spot

Determine co-localization or association index between each RNA and RBP

**c  RBP-driven granule assembly**

Shared RBP

Heterotypic transport granules

RBP–RBP interactions

Stress granules

LLPS by IDRs on RBPs

RBPs with IDRs

Stress granules

**d  RNA-driven assembly**

Specific base-pairing in trans

*bcd, osk* granules

Promiscuous RNA assembly
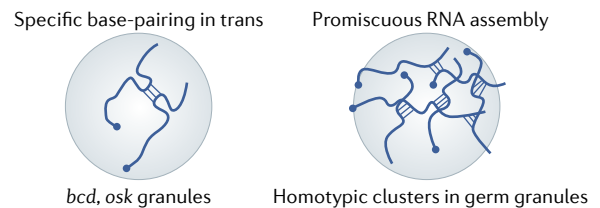
Homotypic clusters in germ granules

Fig. 5 | **Granule composition and organization. a** | Global analysis of mRNA–protein interactions can be performed with proximity-based labelling techniques using ascorbate peroxidase (APEX) or the biotin ligase BioID assay (also known as BirA). A bait protein is fused to the APEX or BirA enzyme, the latter of which biotinylates proteins within a 10 nm radius. In the presence of biotin or biotin phenol, APEX generates short-lived radicals that covalently react with tyrosine and other electron-rich amino acids as well as with amino groups on guanosine. The biotinylated proteins and RNAs are enriched by streptavidin pull-down and identified by mass spectrometry and RNA sequencing (RNA-seq), respectively. **b** | Imaging-based approaches such as single-molecule fluorescence in situ hybridization (smFISH) combined with immunofluorescence (IF) allow users to simultaneously detect individual mRNAs (using labelled oligonucleotides) and their interacting RNA binding protein (RBP; using antibodies) in situ. By precisely localizing these molecules inside cells and registering the point spread functions (PSFs) of the spots, quantitative measurements of mRNA–protein association are obtained. **c** | During RBP-driven granule formation, RBPs are shared between multiple mRNAs at the same time, leading to the packaging of these transcripts into heterotypic granules. RBPs engage in two key forms of protein–protein interactions, namely stereospecific interactions between well-folded proteins (such as other RBPs or G3BPs in stress granules) and/or interactions via intrinsically disordered regions (IDRs) of RBPs. The IDRs in turn specifically interact with well-folded proteins and/or undergo non-specific interactions with proteins in their vicinity to form liquid–liquid phase separation (LLPS) condensates or granules. **d** | RNA–RNA interactions can also promote granule assembly. Specifically, in *trans* interactions between RNAs, such as *bcd* and *osk* mRNAs in *Drosophila melanogaster*, transport granules. Furthermore, the nature of RNAs to self-assemble may promote promiscuous RNA–RNA interactions that assemble mRNAs, in a dose-dependent manner, into stress granules or germ granules.

multiple mRNPs indiscriminately such as into stress granules (discussed below) (FIG. 5c). An alternative model is RNA-driven granule assembly, which favours RNA as the primary scaffold for binding several multivalent RBPs, therefore promoting the nucleation of phase-separated assemblies[216–218]. For example, stress granule assembly is initiated by the stable aggregation of untranslated mRNAs into the core, potentially increasing the concentration of RBPs and therefore driving LLPS[216] (FIG. 5d). Additionally, mRNAs engage in both promiscuous[219] and specific RNA–RNA interactions[220], which is the main driving force for the assembly of stress granules (reviewed in REF.[221]) (FIG. 5d). This non-specific mechanism is determined by the length of the mRNA (the longer the mRNA, the more interaction sites are available for random base-pairing). In *D. melanogaster* germ granules, mRNAs can self-assemble and position themselves in granules in a dose-dependent manner[222].

### Homotypic versus heterotypic granules

The self-assembly of RNAs suggests that RNA granules could be homotypic, constituting the same RNA species, or heterotypic granules formed by mRNAs undergoing base-pairing in *trans*. *D. melanogaster* germ plasm contains granules of up to 500 nm in size, the functions of which are determined by the localization and organization of mRNAs within the granule. During oocyte development, specific base-pairing between *osk* mRNAs or *bcd* mRNAs promotes the recruitment of both mRNAs to granules, rendering them heterotypic[220].

Although germ granules are heterotypic, multiple copies of mRNAs from the same gene are organized into discrete homotypic clusters that occupy specific positions within the granule[222,223]. Interestingly, this self-sorting of clusters within the granules is independent of sequence specificity[222,223].

Stress granules and P-bodies are more heterogeneous in their structure and composition. Transcriptome analyses from yeast to mammalian cells have found that >99% of mRNAs enter stress granules; however, only 10% of these mRNAs are stably located within the granules, and there is a preference for longer transcripts[224]. Although single-molecule imaging has provided tremendous insights into how mRNAs enter and localize in stress granules[225], it remains unclear whether each RNA species forms distinct clusters. Similarly, RNA stoichiometry and organization inside transport granules in mammalian neurons are heavily debated. Results from smFISH against several dendritically localized mRNAs indicated that each mRNA species existed as a single, independent mRNP along dendrites[226] and may not belong to larger granules. Real-time imaging of endogenous *actb* mRNAs in *X. laevis* showed that, in axons, these mRNAs commonly travel as single mRNAs in granules[94] whereas, in dendrites, the dynamic merging and splitting of mRNPs have been observed, suggesting the presence of a mixed population of granules containing one or multiple *ACTB* mRNAs[92]. Interestingly, real-time imaging of two different mRNAs, *Actb* and *ARC*, did not exhibit co-trafficking or coalescing even in crowded dendritic segments (S.D. and R.H.S., unpublished work), furthering the idea that transport granules may be homotypic.

Therefore, several studies suggest that mRNAs might be inherently biased to form homotypic clusters, courtesy of their self-assembling nature. One major advantage of each mRNA species forming its own granule would be the ability to fine-tune the local translation of specific mRNAs without affecting the status of other localized mRNAs. However, heterotypic granules may facilitate the clustering of different mRNAs of multi-subunit protein complexes and promote the co-regulation of their translation according to cellular needs.

### mRNAs in stress granules and P-bodies

Cells adapt to adverse conditions by reprogramming translation, primarily by repressing translation initiation in response to stressors such as increased temperature (heat-shock) and nutrient deprivation[207]. Stressors preclude the formation of the pre-initiation complex by inducing the phosphorylating eIF2α and/or by inhibiting the assembly of the cap-binding complex eIF4F. Untranslated mRNAs are quickly and reversibly rearranged with proteins into stress granules and P-bodies[227], although stress granule formation can be independent of eIF2α phosphorylation[228]. Stress granules and P-bodies are heterotypic mRNP granules enriched in translation and decay factors, respectively[229]. Although stress granules are formed under stress conditions, P-bodies exist in non-stressed mammalian cells and increase in size and number upon stress[230]. These granules are formed by LLPS, which requires both proteins and

RNAs[217,231–235], and they can associate with each other during stress. Characterization of the protein composition of stress granules and P-bodies has defined their common features such as the presence of intrinsically disordered regions and RNA-binding activity. These granules lack substrate specificity, with 20% of cellular RNAs localizing to both of them indiscriminately[224,236]. The release of mRNAs from stalled ribosomes by components of the quality control machinery results in the fast influx of these mRNAs into stress granules, and the inter-molecular interactions that occur facilitate the partitioning of mRNA into condensates[237]. Interestingly, these granules are enriched in long mRNAs with highly structured 5′UTRs and AU-rich 3′UTRs, which accounts for their low stability and translatability[218,238–240].

Recent single-molecule approaches have attempted to visualize the conformation of mRNA within stress granules and P-bodies of mammalian cells as well as their dynamic molecular interactions in vivo[225,241,242]. The molecular compaction of mRNAs was calculated by measuring the distance of the fluorescence signal using smFISH probes against the 5′ coding sequence and 3′ ends of the same mRNA[241,242]. Actively translated mRNAs were in an extended conformation as a result of ribosome loading and compacted up to 200-fold upon the stress-induced inhibition of translation (namely, in response to oxidative stress or heat-shock). This compaction after ribosome run-off occurred in the cytoplasm and usually preceded the localization of mRNA to stress granules, indicating that mRNAs are translationally repressed before entering the stress granules[241]. One caveat to using smFISH methods to assess conformation is that the analysis is restricted to long transcripts (to allow the hybridization of multiple probes); furthermore, compactness may be a product of fixation artefacts. A recent live imaging study visualizing translating mRNAs reported similar stretching of mRNAs upon their increased association with ribosomes in the cytoplasm, validating the observations made in fixed cells[201]. Real-time imaging also revealed that mRNAs engage in both transient and stable interactions with stress granules and P-bodies and move bi-directionally between them[225,243]. The nature of the interaction depends on the size of the P-bodies and stress granules as well as on the length and translational status of mRNA (untranslated mRNAs showed stable interactions, whereas translated mRNAs had sporadic interactions). Larger granules retained mRNAs for extended periods of time, as shown for long transcripts, such as that encoding p300 (~8.3 kb), or mTOR-sensitive transcripts, specifically those bearing a terminal oligopyrimidine tract in their 5′UTR[225,243]. Upon stress withdrawal, stress granules rapidly disappear, and translation is resumed for all kinds of mRNAs trapped inside granules[243]. Although stress granules are widely believed to contain non-translating mRNAs, a recent single-molecule imaging study challenged this notion, demonstrating that translation continues even after the mRNAs are recruited to stress granules from cytosol during acute cellular stress[244]. This phenomenon is not limited to mRNAs whose translation is upregulated during stress, but is also true for transcripts that are inhibited during stress. Therefore, deciphering

the composition of these granules would provide insight into their transcriptome and could reveal whether all stress granule-associated transcripts are similarly regulated. Recently developed proximity labelling approaches such as APEX-seq are powerful approaches towards understanding the constituents and organization of stress granules[212,245]. Although a fluid interchange of components with the cytoplasm occurs in both stress granules and P-bodies, their assembly dynamics, composition and intermolecular interactions are cell-type and stress-type dependent[228,236,245,246]. Likewise, the fate of mRNAs after interacting with P-bodies and stress granules and whether this fate leads to homeostatic reset or pathological outcome depend on the cell type and type of stress. Overall, mRNAs play an essential role in the biogenesis of granules[235]; nonetheless, the functional relevance of mRNA interactions with stress granules or P-bodies in cell survival during stress or in disease progression is still uncertain. Future work should determine the stress-dependent and context-dependent structural and functional diversity of stress granules and P-bodies.

## Conclusion and future perspectives

The localization of mRNAs in subcellular compartments has emerged as a highly conserved mechanism for the spatial and temporal control of protein synthesis. The development of imaging and sequencing-based methods to detect mRNA and translation has made it possible to measure this post-transcriptional regulation across species and cellular scales. Single-gene studies have characterized the molecular events controlling the cytoplasmic localization of mRNA and have started to elucidate the mechanisms of local translation, for instance, at the ER, mitochondria or synaptic sites in dendrites. Large-scale studies have characterized up to thousands of localizing mRNAs and offered a global view of the shared features of trafficking (for example, zipcode elements, functional relationships and the binding sites of RBPs). The impact of mRNA trafficking on the physiology of multicellular tissues and of entire organisms (for example, *C. elegans* and *D. melanogaster*) has unravelled how mRNA localization influences high-order cell-to-cell interactions that are important for tissue organization and homeostasis.

Three major challenges must be overcome to achieve a clear understanding of the molecular events that regulate localized translation. First, few methods are currently available to modulate mRNA localization in vivo for functional studies. Most of these approaches target RBPs or zipcodes and require irreversible loss of function. Recently, the dynamic control of cytoplasmic mRNA localization and possibly translation has been feasible using either the MS2 (REFS[92,133,167,171,247]) or the Cas9 systems[247]. However, these multicomponent systems require complex experimental manipulations that may be far from physiological conditions and difficult to reproduce. Second, the development of in situ transcriptomic technologies generated an overwhelming amount of mRNA localization data to be analysed and interpreted. Several groups are at the forefront in the challenge of developing algorithms that define 'ground-truth' mRNA localization patterns that can be used as a reference for automatic identification in large datasets[156,248–250]. Further automation of these machine learning-based tools will allow us to extract meaningful localization patterns from the vast information generated by multiplex in situ transcriptomic data. Finally, the mRNA localization field constantly benefits from developments in imaging such as brighter and more photostable fluorescent proteins and fluorophores and detectors with an increased sensitivity. These improvements will be key to developing RNA imaging reporters for the detection of endogenous unmodified mRNAs at single-molecule resolution. Biophysicists, chemists and biologists will bring this field one-step closer to understanding how the spatial organization of biomolecules in single cells participates in translating a genotype into a phenotype.

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## Author contributions

S.D., V.G., E.T. and M.V. researched data for the Review; S.D., R.H.S., E.T. and M.V. made a substantial contribution to discussion of content; all of the authors wrote the article and S.D., R.H.S. and E.T. reviewed and edited the manuscript before submission.