

In the right place at the right time: visualizing and understanding mRNA localization

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Abstract | The spatial regulation of protein translation is an efficient way to create functional and structural asymmetries in cells. Recent research has furthered our understanding of how individual cells spatially organize protein synthesis, by applying innovative technology to characterize the relationship between mRNAs and their regulatory proteins, single-mRNA trafficking dynamics, physiological effects of abrogating mRNA localization *in vivo* and for endogenous mRNA labelling. The implementation of new imaging technologies has yielded valuable information on mRNA localization, for example, by observing single molecules in tissues. The emerging movements and localization patterns of mRNAs in morphologically distinct unicellular organisms and in neurons have illuminated shared and specialized mechanisms of mRNA localization, and this information is complemented by transgenic and biochemical techniques that reveal the biological consequences of mRNA mislocalization.

Spatial segregation of protein synthesis in cells involves the positioning of mRNAs according to where their protein products are required, and results in local or compartmentalized gene expression. This asymmetrical distribution of mRNA, termed mRNA localization, can be more thermodynamically efficient than transporting proteins because fewer mRNA molecules need to be mobilized. It is also possible that spatially controlling translation offers finer control of local protein activity¹ as opposed to preventing ectopic activity by other means. Furthermore, proteins synthesized locally are structurally and functionally distinct from transported proteins: they are more likely to contain domains that promote protein–protein interactions, and are subject to tighter regulation of expression and to more post-translational modifications than proteins that are not translated locally¹.

mRNA localization can occur during specific stages in development, and distinguish cell and tissue phenotypes, activities and communication. Recent advances in single-molecule RNA imaging in live cells and whole organisms, as well as advances in genome-wide analyses of RNA–protein interactions, have improved our understanding of how mRNA localization to sub-cellular regions is regulated and accomplished on the single-molecule level. Early visualization experiments of asymmetric mRNA distribution in model

systems — such as ascidian eggs², fibroblasts³, *Xenopus laevis* oocytes⁴, *Drosophila melanogaster* embryos⁵, *Saccharomyces cerevisiae*⁶ and neurons⁷ revealed that dynamic cellular and subcellular mRNA localization is a conserved phenomenon. More recently, it was shown that during *D. melanogaster* development up to 70% of mRNAs are expressed in distinct spatial patterns⁸. Similarly, half of the neuronal mRNA species in the rat hippocampus are enriched in axons and dendrites compared with the cell body (the soma)⁹. Early work uncovered fundamental regulatory mechanisms underlying mRNA localization such as the importance of the cytoskeleton^{10–14} and of conserved *cis*-acting sequences^{15–21}. Identification of mRNA elements responsible for localization preceded the determination of the RNA-binding proteins (RBPs)^{22–24} and molecular motors that mediate transport on the cytoskeleton²⁵. More recently, model systems such as fibroblasts, neurons, budding yeast, *D. melanogaster* oocytes and embryos, and *X. laevis* oocytes have been used to investigate the kinetics and regulation of mRNA movement and localization²⁶, as well as the role of mRNA localization in many aspects of life, such as cell migration, development, neural signalling and disease.

In this Review, we first provide an overview of these cutting-edge techniques, followed by a discussion of the mRNA properties, protein complexes and the cellular

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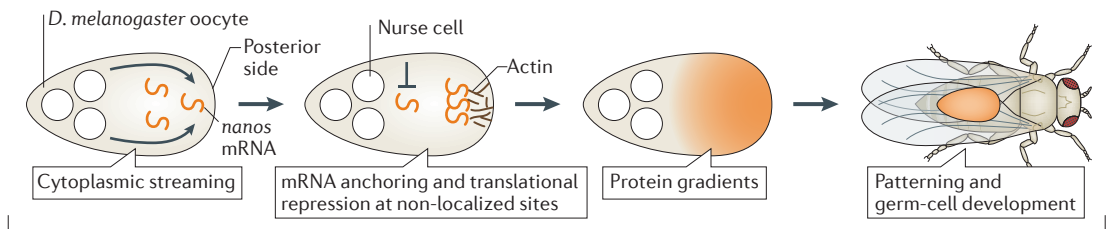
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mechanisms that mediate mRNA localization. We then discuss how the visualization of mRNAs has yielded valuable information on the dynamic behaviour of mRNAs and their transport partners in various cellular processes, with a particular emphasis on mRNA cytoplasmic localization in the unicellular organism *S. cerevisiae* and in neurons.

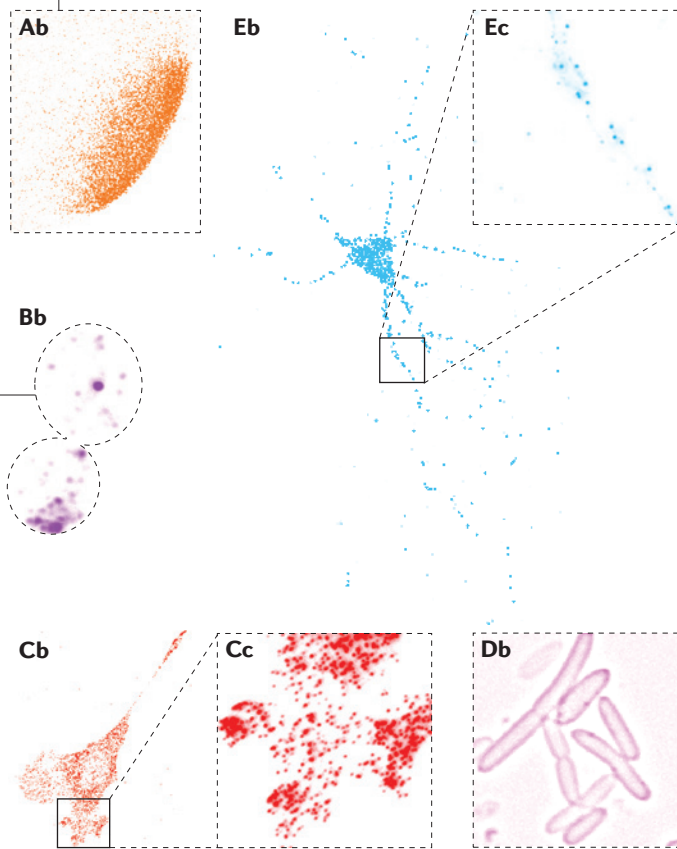
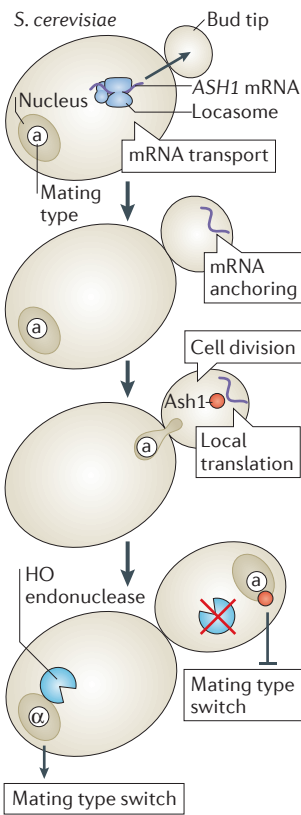
Visualizing the message

Optical techniques have been frequently used to investigate how mRNA localization is accomplished and to identify the factors involved. Below, we discuss methods that are particularly useful for investigating mRNA distribution and dynamics, and highlight discoveries that were made using these approaches.

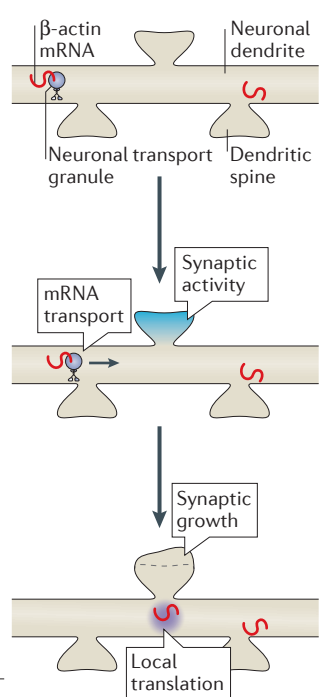
Aa Patterning and development



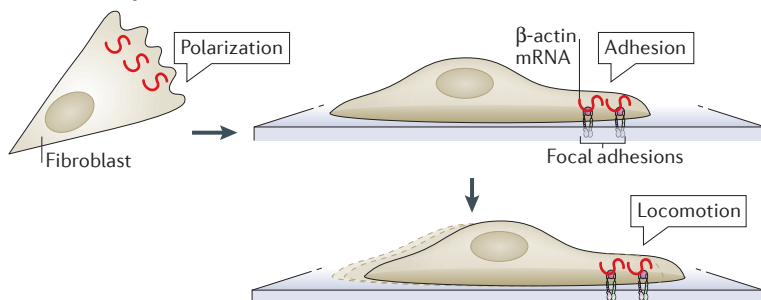
Ba Cell fate determination



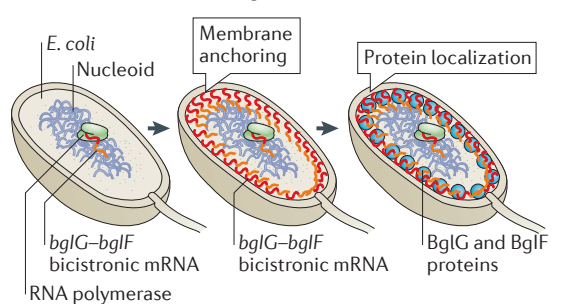
Ea Cell fate determination



Ca Cell motility



Da Membrane anchoring



Single-molecule FISH (smFISH). A fluorescence *in situ* hybridization (FISH) technique that uses multiple unique short probes against a single mRNA, which greatly increases signal-to-noise ratio and enables detection of single mRNA molecules.

SNAP tag

A protein fusion tag derived from the human enzyme O⁶-methylguanine DNA methyltransferase. The protein can covalently bind to a synthetic chemical ligand that can be labelled with a fluorescent dye.

Aptamers

Short nucleic acid sequences with unique folding properties that can bind to a specific target molecule and be used for fluorescent tagging.

In situ hybridization techniques. *In situ* hybridization (ISH) is a method by which labelled, short nucleic acid probes are hybridized to RNA or DNA in fixed cells or tissues. ISH with biotinylated or radioactive probes enabled the first visualization of asymmetrically distributed poly(A), histone and actin mRNAs in muscle cells²⁷ and ascidian eggs². The technical variables of ISH were quantitatively optimized three decades ago²⁸. However, the technique has since improved owing to technological advances, which allowed the development of fluorescence *in situ* hybridization (FISH). The synthesis of fluorescent probes has become exponentially cheaper and more commercially available, fluorescent labels have become brighter, and image detection has become more sensitive, allowing the detection of single mRNAs. In single-molecule FISH (smFISH), multiple fluorescent probes are hybridized to a single mRNA, which enables single-molecule detection without the need for sophisticated imaging instrumentation^{29,30}. Currently, smFISH is easily achievable and has been developed to be rapid³¹, multiplexed^{32,33}, automated and even high throughput³⁴ (FIG. 1; see [Supplementary information S1,S2](#) (figure, table)). Many alternative FISH protocols have been developed for detecting mRNAs, and these mostly differ by the type of probe used (see [Supplementary information S2](#) (table)). To complement mRNA visualization by FISH, computational tools that analyse FISH data in an unbiased manner can provide quantitative insights into mRNA localization (BOX 1).

mRNA imaging in live cells. Much has been gained from FISH studies on how mRNAs are localized in cells; however, dynamic information on mRNA movements was lacking. To overcome this limitation, earlier as well as more recent studies have taken advantage of the binding of RBPs to specific mRNAs, by expressing GFP–RBP chimaeras as a way to indirectly follow mRNA dynamics^{35–37}. A substantial advancement in mRNA imaging was the use of direct fluorescent tagging of mRNAs using the MS2 bacteriophage system. In this method, the bacteriophage MS2 coat protein (MCP) binds to a unique RNA hairpin sequence (MS2-binding site (MBS)) that can be cloned into the mRNA of choice^{38,39}. Multimerization of the MBS stem-loops and co-expression of MCP fused to a fluorescent protein (MCP–FP) enables time-lapse imaging of mRNA kinetics and localization in live cells (FIG. 2a).

Homologous systems using cognate hairpin-coat proteins were developed (see [Supplementary information S2](#) (table)). The U1A mRNA labelling system, which is limited to non-mammalian cells, uses the RNA–protein couplet of the human U1A protein, a component of the spliceosomal U1 small nuclear ribonucleoprotein (U1 snRNP), and a specific RNA hairpin⁴⁰. Similarly, the phage PP7 coat protein (PCP) and its cognate RNA hairpin were cloned⁴¹, as was the λ -phage N-protein–boxB system⁴², allowing live-cell imaging of two mRNA species simultaneously^{42,43} (FIG. 2b). There are ongoing improvements in coat protein labelling of mRNA secondary structures. For instance, as MCP dimerization is a prerequisite for binding to the MBS, expression of a genetically dimerized version of MCP increases mRNA binding efficiency⁴⁴. Furthermore, the use of a bimolecular fluorescence complementation system — whereby different coat proteins are fused to a split fluorescent protein such that only the binding to mRNA stem-loops will form a competent fluorescent protein — results in ‘background-free’ imaging⁴⁵ (FIG. 2c). To complement these live mRNA imaging methods, computational particle-tracking tools (BOX 1) are used to analyse mRNA movements in the cell.

Other techniques use exogenous fluorescent dyes to label RNAs in live cells. These dyes bind either to a dedicated protein (for example, a SNAP tag⁴⁶) fused to a coat protein or directly to RNA aptamers (such as Spinach and RNA-Mango^{47,48}). These dyes can be brighter and more photostable than fluorescent proteins. However, delivery of non-genetically encoded labels may be more detrimental to the cell and may introduce background fluorescence. Furthermore, some of the RNA aptamers may not be suitable for single-molecule imaging (see [Supplementary information S2](#) (table)).

In addition to labelling for imaging purposes, the interactions of coat proteins with mRNAs are used to alter intracellular mRNA localization. MCP fusions to various cellular components have enabled the artificial localization of mRNAs to subcellular sites to rescue mRNA localization defects⁴⁹ (FIG. 2d). Other uses of MS2–MCP-like systems include the affinity purification of RNAs (FIG. 2e), tethering proteins to the mRNA⁵⁰ (FIG. 2f) and the simultaneous localization of mRNAs and their protein products⁵¹ (FIG. 2g).

◀ **Figure 1 | Visualizing and understanding mRNA localization in different model systems.** **Aa** | mRNA localization in oocytes and embryos can be essential for future patterning and development. In *Drosophila melanogaster* oocytes, cytoplasmic streaming from the nurse cells at the anterior drives *nanos* mRNA to the posterior, where it is anchored⁹ and translated, whereas mRNAs not present at the posterior are repressed to prevent translation¹⁷³ (see BOX 2 for a discussion on another mechanism contributing to the localized translation of *nanos* mRNA). **Ab** | Single-molecule fluorescence *in situ* hybridization (smFISH) of *nanos* mRNA localization in a 1-hour-old *D. melanogaster* embryo is shown. **Ba** | mRNA localization has an important role in cell fate determination during cell division. For example, in *Saccharomyces cerevisiae*, *ASH1* mRNA, which encodes a transcription inhibitor, is transported to the bud tip by the localosome, a protein complex consisting of several RNA-binding proteins (RBPs) and a myosin motor. Local translation at the bud tip inhibits the transcription of the homothallic switching (HO) endonuclease, which is required for mating type switch, thus preventing mating type switching in the daughter cell. **Bb** | smFISH of *ASH1* mRNA in *S. cerevisiae* is shown. **Ca** | mRNA localization and local translation in fibroblasts have been shown to be important for proper cell migration and motility. For example, β -actin mRNA localization to the cell edge is correlated with cell polarization, and β -actin mRNA localization to focal adhesions is crucial for proper cell migration⁴⁹. **Cb** | smFISH of β -actin mRNA in a cultured mouse embryonic fibroblast is shown. **Cc** | Enlarged image of dashed box in part **Cb** is shown. **Da** | In *Escherichia coli*, the bicistronic *bglF–bglG* transcript localizes to the plasma membrane in a translation-independent manner. This leads to the localization of BglF and BglG to the membrane. If *bglG* is transcribed as a monocistronic mRNA, it will localize to the poles (FIG. 4B) where, under certain conditions, the BglG protein will also be localized. **Db** | Image shows membrane-localized MS2–GFP-tagged endogenous *bglF* mRNA in *E. coli*. **Ea** | In neurons, mRNA localization to synapses is thought to be crucial for synapse development and plasticity. mRNAs, such as β -actin, are transported in dendrites, and synaptic activity is proposed to cause mRNAs to localize at stimulated synapses. The local translation of β -actin is proposed to cause morphological and functional alterations of synapses. **Eb** | smFISH of β -actin mRNA in a cultured mouse hippocampal neuron is shown. **Ec** | Enlarged image of dashed box in part **Eb** is shown. Each side of the dashed boxes of parts **Ab**, **Cc** and **Ec** represents 20 μ m. Image in part **Ab** courtesy of T. Trcek and R. Lehmann, New York University School of Medicine, USA. Image in part **Bb** courtesy of T. Trcek. Image in part **Db** courtesy of O. Amster-Choder, The Hebrew University of Jerusalem, Israel.

Box 1 | Quantitative analysis tools of mRNA localization

The advent of the visualization of single mRNA molecules in individual cells necessitated the unbiased quantification of their abundance, distribution and movement in a variety of cell types. The following are some quantitative tools for analysing single mRNA molecules in cells.

Analysis of fluorescence *in situ* hybridization spots

Many laboratories specializing in single-molecule fluorescence *in situ* hybridization (smFISH) write their own analysis tools, some of which are based on IDL^{154,155} or MATLAB^{53,156} programs. Most programs use two-dimensional Gaussian fitting of candidate spots to obtain a sub-diffraction localization of single mRNAs¹⁵⁶.

The freeware [FISH-quant](#) can automatically analyse both cytoplasmic (single) mRNAs and nascent transcripts at transcription sites in three dimensions¹⁵⁶. With smFISH, it is not always straightforward to prove that one is imaging the signal of a single mRNA, especially as multiple probes are used for their detection. To this end, the intensity distribution of FISH spots analysed with two- or three-dimensional Gaussian fitting should exhibit a single Gaussian distributed peak. In some cases, the number of probes bound to a single mRNA may be calculated by dividing FISH spot intensity by the intensity of a single FISH probe^{29,62}.

Analysis of mRNA localization

The visual determination of the extent of mRNA localization in a cell is essential for studying mRNA localization qualitatively; however, these studies typically lack a quantitative element so they are subject to human bias. To automate the quantification of mRNA localization, an unbiased analytical method was developed to objectively quantify cell polarization based on the distribution of the β -actin mRNA¹⁵⁷.

Single-particle tracking

Tracking single mRNAs in live cells has been instrumental for gathering information on the mechanisms of mRNA localization²⁶. Many of the principles of single-molecule identification and detection used for analysing FISH data (which are obtained from fixed cells) apply to live-cell imaging, although deteriorated signal-to-noise ratio, rapidly moving particles and temporary particle disappearance are some of the many challenges of tracking molecules in live cells. Tracking algorithms use various computational methods to link particles between successive frames^{158,159}. Many tracking tools are freely available¹⁶⁰, and a popular one is u-track¹⁵⁹. An exhaustive comparison of the tracking methods used by 14 different research groups was recently published¹⁶¹.

Fluorescence correlation spectroscopy. Imaging single mRNA molecules has brought with it a need to acquire absolute measurements of the concentration, movement, interactions and specific composition of a single mRNA–protein (mRNP) complex. One method to do this is fluorescence correlation spectroscopy (FCS), a powerful technique to directly measure diffusion, concentration and molecular interactions of single molecules *in vitro* and *in vivo*. This technique measures the fluctuations in fluorescence intensity of fluorescently labelled molecules, which result from their diffusion through a sub-femtolitre observation volume. Fluctuation analysis has allowed the measurement of subcellular, local diffusion properties and local concentrations of MCP-labelled endogenous mRNAs⁴⁴, transcription factors and mRNA production rates⁵². Furthermore, brightness and diffusion measurements can reveal mRNA aggregation or dimerization, and dual-colour imaging of a single mRNA in conjunction with fluorescently labelled RBPs allows investigators to precisely quantify the association of the two species through fluorescence cross-correlation spectroscopy (FCCS).

Transgenic organisms for mRNA imaging. Transgenically modifying mRNAs to enable their fluorescent labelling circumvents many challenges of expressing exogenous

mRNA tags and supports physiological mRNA metabolism. The development of genetically encoded systems to tag endogenous mRNAs was initially used in yeast, which is highly amenable to genetic manipulations. Transgenic model systems of higher complexity have allowed the visualization of endogenous mRNA localization in primary cells⁵³, oocytes^{54–56}, embryos⁵⁷, tissue slices⁵⁸ and even whole animals⁵⁸. MS2 stem-loops and fluorescent coat proteins expressed in transgenic *D. melanogaster* have increased our understanding of the localization mechanisms of endogenous *nanos*⁵⁷, *bicoid*⁵⁶, *oskar*⁵⁴ and *gurken*⁵⁵ mRNAs. The first mammalian transgenic animal for imaging mRNA in living tissue was a mouse with MBS inserted into the 3' untranslated region (UTR) of the β -actin mRNA, which allows its imaging in every cell of the animal⁵³. Subsequently, a transgenic mouse expressing MCP fused to GFP was crossed with the β -actin–MBS mouse, bypassing the need to deliver MCP and enabling global fluorescent labelling of the endogenous β -actin mRNA for direct imaging in cells and in tissues⁵⁸. The advent of CRISPR–Cas9 (clustered regularly interspaced short palindromic repeat–CRISPR-associated protein 9) tools for carrying out genetic modifications will expedite the generation of transgenic animals for the imaging of endogenous mRNAs⁵⁹. However, as many mRNPs contain only a single mRNA^{29,60–64}, contesting tissue autofluorescence in certain circumstances may require brighter labels for *in vivo* mRNA imaging of single molecules.

Cellular mechanisms of RNA localization

Overcoming entropy to maintain asymmetry in cellular mRNA distribution requires the orchestration of many mRNA adaptors and regulators. The transport, translation, protection from degradation and anchoring of mRNAs are all determined by RBPs. In turn, the interaction of RBPs with mRNAs is determined by the localization elements in the mRNAs that operate like subcellular localization 'zip codes' (BOX 2; reviewed in REFS 65,66) or, in certain instances, by the gene promoter⁶⁷. To spatially restrict translation, mRNA distribution must be accompanied by translational repression while the mRNA is in transit (reviewed in REF. 68). Interestingly, many RBPs simultaneously maintain translational repression while coordinating mRNA trafficking (reviewed in REF. 69).

mRNA–protein complexes form granules. mRNAs and proteins are organized in cellular units of diverse composition, structure, size and function, all of which are loosely termed RNA granules. To understand how mRNA localization is regulated, emphasis should be placed on the characterization of the specific composition, size and diversity of granules of unique mRNAs. There are many well-understood examples of mRNA–RBP interactions studied outside their cellular context; however, the higher-order structure and make-up of mRNP complexes and granules *in vivo* are still poorly understood. Sequence binding specificity of any mRNA–RBP pair can be defined down to the nucleotide level, paving the way for identifying other putative mRNAs

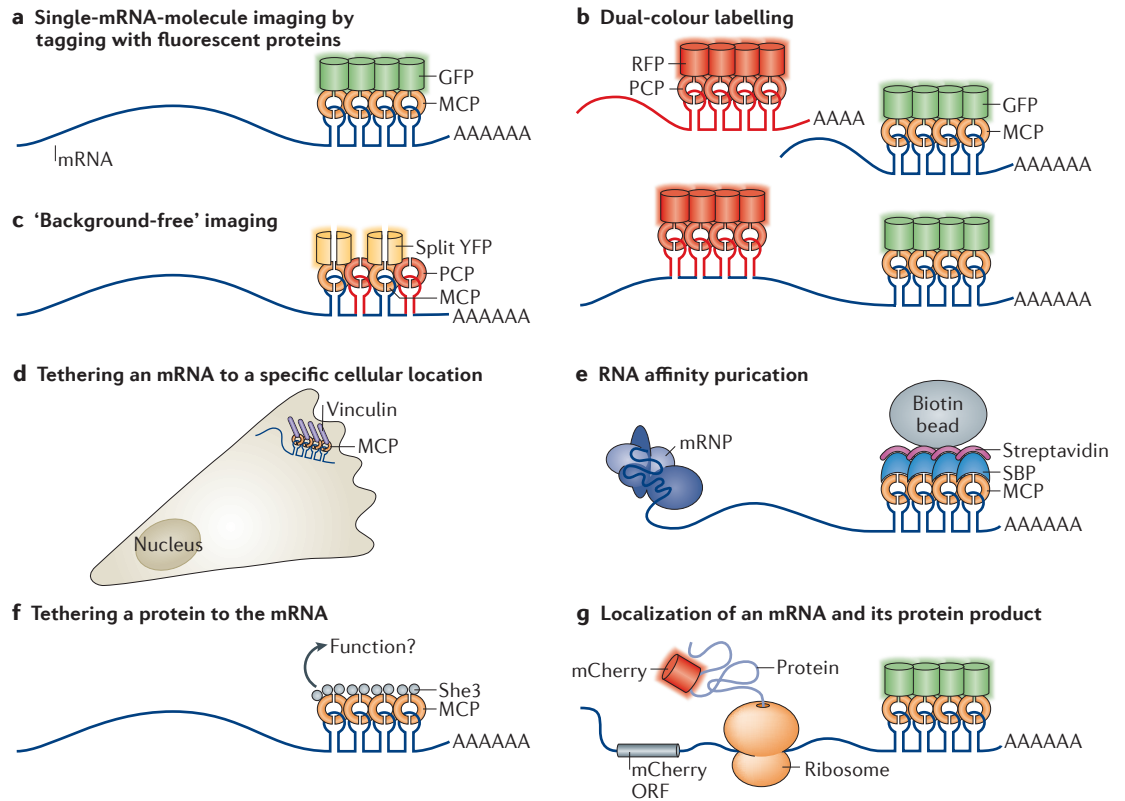


Figure 2 | Traditional and novel uses of MS2-like systems to investigate mRNA biology. **a** | Localization of single mRNA molecules can be studied by tagging with fluorescent proteins. The fusion of a stem-loop-binding protein, for example, the phage MS2 coat protein (MCP), to a fluorescent protein such as GFP allows single-molecule mRNA imaging^{38,39}. **b** | In dual-colour labelling, two mRNAs (top) or two different parts of the same mRNA (usually the 3' and the 5'; bottom) are tagged by different stem-loop-RNA-binding protein (RBP)-fluorescent protein systems, thereby allowing the imaging of two different mRNAs in the same cell or the analysis of RNA dynamics, such as transcription, nuclear export and degradation⁴³. **c** | A 'background-free' system is shown. To reduce background fluorescence, two different stem-loop species (for example, those of the MS2 and PP7 phage systems) bound to their respective RBPs — MCP and PP7 coat protein (PCP) — are used. Each RBP is fused to one half of a split yellow fluorescent protein (YFP), which by itself does not fluoresce. Only when both MCP and PCP are bound to the mRNA are the two halves close enough to become a functional YFP⁴⁵. **d** | The tethering of an mRNA to a specific cellular location or structure (for example, focal adhesions) is carried out by fusing MCP to a protein (in this case, vinculin) with specific subcellular localization that anchors the mRNA to a specific cellular compartment, body or organelle⁴⁹. **e** | In RNA affinity purification, an RBP such as MCP is fused to a unique epitope — for example, streptavidin-binding protein (SBP) — which mediates the affinity purification of the RNA (along with mRNA-protein (mRNP) complexes that might bind to it) using streptavidin and biotin beads¹⁴⁹. **f** | A specific protein can be tethered to an mRNA through RBPs. The protein in question, which is thought to affect the mRNA or its associated proteins (for example, the transporter She3), is fused to MCP, which tethers it to the mRNA and allows the analysis of this direct interaction⁵⁰. **g** | Simultaneous localization of the mRNA and its protein product can also be studied. By fusing the gene to the mCherry (a red fluorescent protein) open reading frame (ORF) and cloning MS2-binding sites (MBSs) into its 3' untranslated region (UTR), the mRNA can be visualized by MCP-GFP binding and the protein by mCherry fluorescence⁵¹. See Supplementary information S2 (table) for more information.

bound by similar RBPs⁷⁰. As a single type of RBP may have hundreds of mRNA targets⁷⁰⁻⁷³, we may assume that perhaps at least dozens of RBPs and RNA regulatory proteins interact with a single mRNA at any given time; for example, *gurken* mRNA is known to be bound and regulated by a number of RBPs^{74,75}. Similarly, β -actin mRNA has so far been shown to interact with at least 10 RBPs^{24,76-82}, although the direct role of some of them in its localization remains to be determined.

Biochemical affinity purification and identification approaches have been instrumental in identifying scores of proteins that are components of various

RNA granules⁸³⁻⁸⁵, but these do not indicate how diverse the composition of individual granules can be. The direct comparison of the proteins associated with the RBPs Staufen2 and Barentsz in RNA granules showed that they only have a 30% overlap, and that their RNA-binding profiles are also enriched with unique mRNAs⁸⁵. This illustrates the potential extent to which granules with diverse mRNA compositions are composed of unique protein components. Interestingly, the assembly of proteins into granules is emerging as a special characteristic of RBPs, and is facilitated and modified by certain RBP motifs^{86,87}. Thus, when studying how RBPs affect

Box 2 | **Cis-acting elements that control mRNA localization**

Short sequences in mRNAs can act in cis to control mRNA localization by one or more of the following mechanisms.

Promoting active and directed transport

The β -actin mRNA ‘zip code’ is a 54-nucleotide (nt) sequence in the 3’ untranslated region (UTR) that contains a bipartite motif, which is recognized by the RNA-binding protein (RBP) zipcode-binding protein 1 (ZBP1)⁷⁰ (see the figure, part a). Binding of ZBP1 to the mRNA is necessary and sufficient to localize it to the leading edge of fibroblasts in a cytoskeleton- and motor-dependent manner^{162,163} (see the figure, part a) and to neuronal dendrites¹⁶⁴. The 3’UTR of the *Drosophila melanogaster bicoid* mRNA contains several 50-nt sequences termed *bicoid* localization elements, which form stem-loop structures that form intermolecular interactions (see the figure, part b). The dimerization of the stem-loops and association of the double-stranded RNA with the RBP Staufen are required for the active transport of *bicoid* mRNA along microtubules at late oogenesis and for its anchoring to the anterior pole^{23,165–167}.

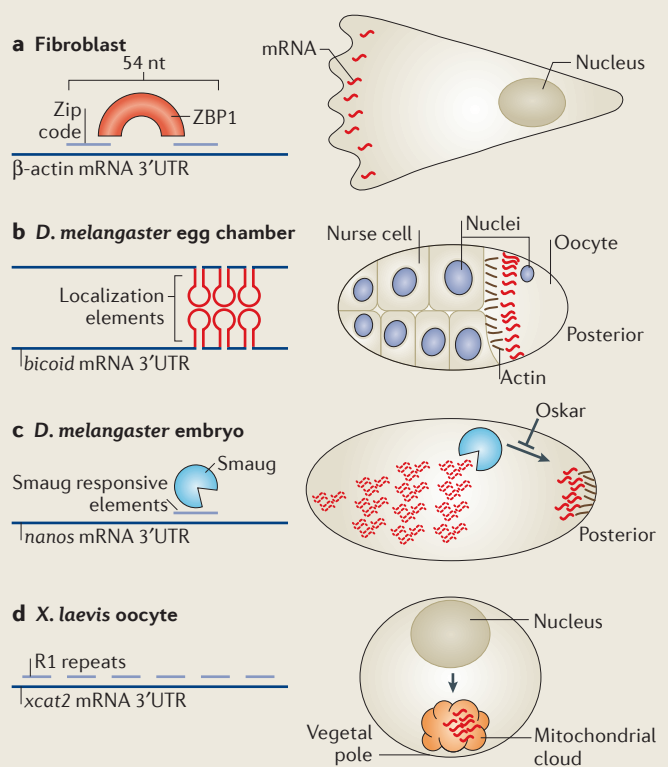
In yeast, the *ASH1* mRNA contains four localization sequences, three in the coding region and one in the 3’UTR. These sequences are required for the association with the local asome and for active transport by the myosin motor Myo4 (FIG. 1B).

Mediating local stability

The mRNA for heat shock protein 83 (*hsp83*) localizes to the posterior pole plasm of *D. melanogaster* embryos. This is accomplished by extensive degradation of this mRNA throughout the cytoplasm, except at the pole plasm. Elements at the *hsp83* 3’UTR were identified as responsible for this protection¹⁶⁸. The *D. melanogaster nanos* mRNA is localized to the posterior pole of the embryo by several mechanisms (FIG. 1A; see below), including through the local stabilization of the transcript by Oskar, which prevents the degradation factor Smaug from binding Smaug-responsive elements in the 3’UTR of *nanos* mRNA¹⁶⁹ (see the figure, part c).

Entrapment and anchoring of diffusing mRNAs

At late *D. melanogaster* oogenesis, the main mechanism for *nanos* mRNA localization is diffusion and entrapment when strong cytoplasmic flows move it throughout the oocyte so it can encounter a localized, actin-based anchor at the posterior pole⁵⁷ (FIG. 1A). This localization is mediated by multiple RBPs, which associate with cis-elements at the *nanos* mRNA 3’UTR^{170,171}. During early oogenesis in *Xenopus laevis* oocytes, *xcat2* and *xdaz1* mRNAs are localized at the vegetal pole in an assembly of mitochondria and entrapped mRNA (called the mitochondrial cloud). Through a microtubule-independent mechanism, the mitochondrial cloud containing the mRNA migrates to the vegetal pole by diffusion (see figure, part d). The 3’UTR of *xcat2* contains six repeats of a short motif, UGCAC (named R1). Point mutations in the second, third or fourth repeats resulted in reduced *xcat2* localization to the mitochondrial cloud. However, the insertion of R1 motifs to the 3’UTR of another mRNA, *vg1*, did not result in its localization to the mitochondrial cloud. Thus, R1 motifs are required but not sufficient for correct mitochondrial cloud localization¹⁷².



mRNA localization, the combinatorial effect of many RBPs on a single mRNA may make it difficult to parse out their individual roles. Conversely, the large number of potential mRNA targets of an individual RBP may obstruct interpretations of how RBPs affect cell physiology through the loss of mRNA localization.

Omnia mea mecum porto: All that is mine, I carry with me. mRNAs often travel as single entities in cells^{29,60–64} and often exhibit distinct localization patterns⁸. This individualistic behaviour may confound

our understanding of how cells accomplish the localization of multiple mRNAs. Unbiased screens in hippocampal brain tissue and *D. melanogaster* embryos identified thousands of localized mRNAs^{8,9}; conversely, only 600–800 mammalian RBPs have been recognized so far (REFS 88, 89; see the [RBP database](#)), raising intriguing questions about the mechanisms of differential and unique localization of multiple mRNAs. As individual RBPs may have tens to hundreds of mRNA targets^{70–73} and a single mRNA may bind to many proteins, it is possible that mRNAs contain unique

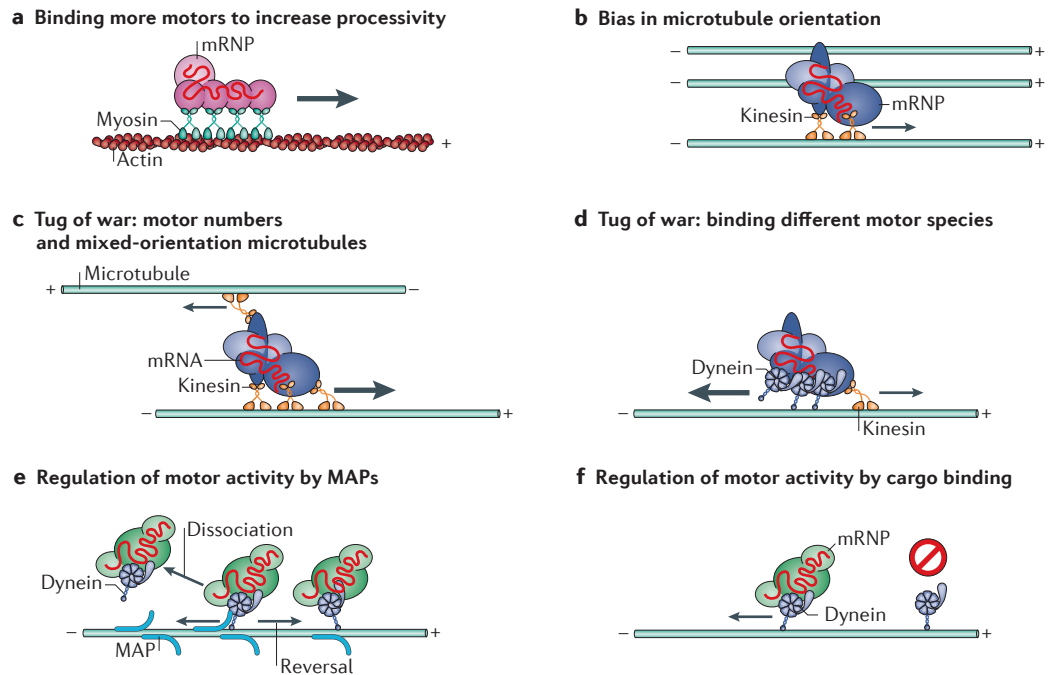


Figure 3 | Cellular determinants of motored mRNA transport. Owing to the bidirectional orientation of microtubules in most cell types and to the unidirectional movement of each molecular motor along them, the directional movement of mRNA in cells may seem disorganized when observed. Nevertheless, several cellular mechanisms of biased motored mRNA transport have recently been identified. **a** | To increase the processivity of directed mRNA transport, some mRNAs bind to multiple motors. For example, each *ASH1* mRNA molecule has four localization elements, which mediate the binding of four She3 RNA-binding proteins (RBPs) and, in turn, the binding of four myosins⁹². **b** | Local biases in the orientation of microtubules have been shown to cause a bias in mRNA transport, allowing mRNA localization^{54,94,176}. **c** | In the case of mixed-orientation microtubules, mRNAs bound to multiple motors may experience a ‘tug of war’ and will be transported in the direction of the strongest combined motor force. **d** | Alternatively, mRNAs bound by different types of molecular motors, which move in opposite directions, may also undergo a tug of war and will be transported in the direction of the stronger force exerted. **e** | Microtubule-associated proteins (MAP) have been shown to alter the dissociation rates of motors from microtubules and to cause a motor to change direction when moving along a microtubule, presumably by behaving as an obstacle^{93,95}. **f** | The binding of cargoes to motors has been shown to alter their binding and motility on microtubules⁹⁸, in addition to increasing their processivity along microtubules^{96,97}. mRNP, mRNA–protein.

Myosin

A family of actin-based, ATP-dependent motor proteins.

Kinesin

A class of molecular motors that use ATP to move along microtubule filaments and that transport many cellular components. There are 14 subtypes in the kinesin superfamily, most of which transport cargo to the plus ends of microtubules.

Dynein

A motor protein family that uses ATP to transport cargo along microtubules, typically towards their minus ends. Axonemal dynein has roles in cilia and flagella, whereas cytoplasmic dynein transports mRNAs, among other cargos.

combinations of sequence elements that dictate their protein associations and thus their subcellular localization (BOX 2; reviewed in REFS 65,66), as well as their metabolism and function. Consistent with this, the stoichiometry of mRNA and RBPs in cells seems to be carefully regulated, as the overexpression of RBPs may exaggerate mRNA localization patterns^{88,89}. Studies investigating mRNA movements in live cells have shown that RBPs bias the stochastic nature of mRNA diffusion and transport, resulting in asymmetric intracellular mRNA distribution²⁶. Below, we discuss examples of the biases RBPs introduce to mRNA behaviour to alter their spatial distribution.

Transport of mRNAs by motor proteins. Active, motor-based locomotion of mRNAs along the cytoskeleton can swiftly transport them throughout the cell (FIG. 3). In fact, an mRNA transported by a molecular motor moving at 1.5 μm per second can transverse the same distance 60 times faster than by diffusion⁶⁰. The motor proteins myosin V, which transports vesicles along actin filaments, as well as certain members of the kinesin and the cytoplasmic dynein families, which transport cargo

along microtubules (mostly in the plus-end and minus-end directions, respectively), all have important roles in actively transporting mRNAs in various biological systems (reviewed in REF. 90). Motored transport of mRNAs is emerging as a complex process that requires the orchestration of many proteins (reviewed in REF. 91).

Although it is known that certain RBPs are necessary for active transport-dependant mRNA localization, few have been shown to interact directly with motor proteins. Transported mRNAs are likely to bind to complexes of RBPs, motors and adaptor proteins. As mRNAs can be transported by multiple motor proteins, multiple RBPs must coordinate their binding and function during the localization process⁶¹. Indeed, the number of localization elements on a single mRNA linearly correlates with the number of motors that bind to it⁹² (FIG. 3a). The increased likelihood of mRNA binding to motors through the inclusion of localization elements and through elevated RBP binding is known to increase the processivity, or run length, of single mRNAs⁶¹ and RBP particles³⁷ along the cytoskeleton (FIG. 3a). Thus, binding to RBPs can improve mRNA transport by increasing the net distance the mRNA travels.

Interestingly, removing a localization element or mutating a regulatory RBP does not necessarily prevent net mRNA translocation; however, on average the paths of active transport are shorter and the probability of a transported mRNA reversing in direction increases^{37,60,61,91}. It has been observed that the *lacZ* mRNA cloned from *Escherichia coli* and expressed in mammalian cells, which has no known localization elements, exhibits low levels of active transport⁶⁰. Similarly, the localized *S. cerevisiae* *ASH1* mRNA (see below) retains a low probability of associating with a myosin motor when stripped of its zip code⁹². The movement of mRNAs devoid of localization elements suggests that there is an intrinsic mechanism for the interaction of mRNAs with molecular motors. Therefore, active transport may allow homogenous intracellular mRNA distribution^{60,93}. These studies imply that active transport is not inherently biased, signifying that the attachment to a motor alone will not localize mRNAs. Therefore, delivering mRNAs to subcellular compartments would require additional mechanisms to regulate the directionality of active mRNA transit.

As microtubules and actin filaments are polarized, directional specificity may be regulated by the differential binding of RBPs and motor proteins to mRNAs. For example, mRNAs localized to the apical cytoplasm in *D. melanogaster* syncytial blastoderms have been shown to be biased towards minus end-directed movement on microtubules owing to the specific binding of Bicaudal D and Egalitarian proteins, which themselves bind to dynein⁸⁹. Similarly, the presence of a localization signal in specific mRNAs increases their processivity and transport towards minus ends of microtubules owing to the increased association with dynein⁶¹. In other cases, the polarity of the microtubules itself is distributed non-randomly within the cell. For example, the localization of *oskar* mRNA to the posterior of *D. melanogaster* oocytes is the result of a kinesin-based active transport with a slight bias in the posterior direction owing to biased microtubule polarity^{54,176}. Likewise, the distribution of mRNAs to the vegetal cortex of *X. laevis* oocytes is accomplished by the enrichment of microtubules oriented with their plus ends positioned at the vegetal cortex⁹⁴ (FIG. 3b).

In many of the aforementioned cases, single mRNAs or RNPs are seen to travel in a bidirectional manner, indicating a simultaneous binding of both kinesin and dynein, which typically traffic to opposite ends of microtubules. Synchronizing multiple motors that exert opposing forces on a single RNP to enable directional travel towards a destination is a complex, regulated process. Simply considering the types and numbers of motors bound to an RNP and the net force of multiple motors pulling in opposite directions should determine the net directionality of mRNA transport (FIG. 3c,d). However, the binding of a motor to an RNP may not linearly contribute to its active transport, as the binding affinity of motor proteins to the cytoskeleton and their motor activity are subjected to regulated modification (reviewed in REF. 95). For example, microtubule-associated proteins can regulate motor dynamics either by altering their microtubule binding and dissociation kinetics or by altering the motility properties of motors, such as increasing reversals in

direction, presumably by acting as obstacles^{93,95} (FIG. 3e). In other cases, adaptor proteins^{96,97} and RBP cargoes^{98,99} have been shown to activate or alter motor activity (FIG. 3f). It is also intriguing that a single mRNA species may be subject to different mechanisms of transport in different cell types; for example, β -actin mRNA in glia cells is largely diffusive, whereas in neuronal dendrites β -actin and *ARC* mRNAs are static or motored^{58,62,100,101} (see [Supplementary information S3](#) (movie)). Although it is tempting to assume that altered mRNA behaviour is the result of disparate RBP expression, enigmatically the same regulatory proteins that localize *nanos* mRNA to the posterior pole of *D. melanogaster* embryos through diffusion and entrapment (BOX 2) have a role in mediating its dynein-dependant transport in larval neurons¹⁰².

mRNA anchoring. In certain cases, mRNA localization may be brought about partially or entirely by spatially selective mRNA capturing or anchoring. Many examples of actin-dependent anchoring of mRNAs have been observed. In fibroblasts, β -actin mRNA is linked to actin filaments via the translational machinery¹⁰³. *vg1* (also known as *dvr1*) mRNA localization in *X. laevis* oocytes depends on microtubule-based transport and actin-based anchoring at the vegetal cortex¹². Actin-dependent anchoring of *nanos* and *oskar* mRNAs was shown to occur in the posterior of *D. melanogaster* oocytes^{57,104} (BOX 2; FIG. 1A). Similarly, anterior localization of *bicoid* mRNA in *D. melanogaster* oocytes depends on active transport of the mRNA followed by actin-dependent anchoring⁵⁶ (BOX 2).

In addition to the actin cytoskeleton, in *D. melanogaster* motors themselves were shown to dock mRNAs after transporting them. For example, dynein converts from functioning as an active motor to behaving as an anchor during the localization of *fushi tarazu* mRNA in blastoderms¹⁰⁵. In yeast, *ASH1* mRNA is anchored at the bud tip following its transport along the actin cytoskeleton (FIG. 1B). Although the mechanism of anchoring is not fully known, it requires specific secondary structures along the open reading frame (ORF) and the active translation of the carboxy-terminal region¹⁰⁶. In neurons, *ARC* mRNA (which encodes activity-regulated cytoskeleton-associated protein) is found anchored underneath individual dendritic spines, or synaptic contacts¹⁰¹, raising intriguing possibilities of synapse-specific modification of transmission properties through local translation.

Localization in unicellular organisms

Asymmetrical cell organization is not restricted to cells of multicellular organisms: both unicellular eukaryotes and bacteria exhibit asymmetries^{107,108}, such as uneven distribution of intracellular organelles or protein complexes, cellular extensions and asymmetric cell division. mRNA localization has been shown to have an important role in generating and maintaining many of these asymmetries in unicellular organisms.

mRNA localization in yeast. In budding yeast, cell division is asymmetrical, such that the daughter cell buds from the mother cell and has a different mating type.

Syncytial blastoderms

A specific stage of *Drosophila* spp. embryogenesis during which the embryo becomes a single multinucleated cell.

Vegetal cortex

The lower pole on the animal vegetal axis of oocytes where the yolk resides.

Bud tip

The point opposite to the bud neck (which connects the bud to the mother cell) in budding yeast.

Mating type

The budding yeast has two mating types, α and a . Mating of a α and a haploid cells produces a diploid cell that can later undergo meiosis to form spores. Haploid cells can switch mating types.

mRNA localization is known to play a crucial part in this process, and the dynamics of mRNA localization have been well characterized in this system. The inaugural use of the MS2 system was to study *ASH1* mRNA in haploid yeast cells to investigate its localization during asymmetrical cell division^{38,39}. During cell division, *ASH1* mRNA and other mRNA species, proteins and organelles are transported to and anchored at the bud tip. Ash1 is a DNA-binding protein that represses the transcription of the homothallic switching (HO) endonuclease in the daughter cell nucleus, thereby inhibiting mating type switching. Localization of *ASH1* mRNA to the bud tip during anaphase causes Ash1 repression of *HO* expression in the daughter cell but not in the mother cell^{6,109–111}, thus inhibiting mating type switching exclusively in the daughter cell (FIG. 1B). FISH and live imaging experiments determined the exact timing at and mechanism by which the *ASH1* mRNA is delivered to the bud tip^{6,38,39}, and identified both *cis* and *trans* factors that are required for its localization (reviewed in REFS 112–114). Briefly, the RBP She2 recruits *ASH1* mRNA to a myosin motor protein (Myo4) via an adaptor protein (She3). This complex, termed the locosome^{38,39}, transports the mRNA along the actin cytoskeleton to the bud tip¹¹¹. The assembly of a functional locosome complex is likely to occur in the nucleus and requires the nuclear pore protein Nup60 (REF. 115). Once in the cytoplasm, the RBPs Khd1 (also known as Hek2) and Puf6 repress the translation of *ASH1* mRNA until it reaches the bud tip^{116,117}. Puf6 is co-transcriptionally recruited to *ASH1* mRNA, as well as to five other bud-tip localized mRNAs, by She2 and another nuclear RBP, Loc1 (REF. 118). Puf6 inhibits translation by binding to eukaryotic initiation factor 5B (eIF5B), which prevents ribosome subunit binding to the mRNA. At the bud tip, the membrane-associated casein kinase Yck1 phosphorylates Puf6 and Khd1 exclusively, leading to the release of eIF5B and enabling translation¹¹⁹.

In a recent study, which used *ASH1* mRNA as a model for mRNA transport, increasing the number of *ASH1* localization elements on single *ASH1* mRNA molecules resulted in a linear increase in the frequency and processivity of its transport, and this represents an important demonstration of how motor cooperativity is used to enhance mRNA localization⁹² (FIG. 3a). Interestingly, the absence of an mRNP cargo abolishes motor protein motility, demonstrating cargo-dependent regulation of motor activity⁹⁸ (FIG. 3f). More recently, an *in vitro* study of *ASH1*–motor complex assembly showed that the She2–She3 complex was required for motor movement but that the mRNA cargo itself was dispensable⁹⁹.

Tagging endogenous mRNAs in yeast with the MS2 system has also helped to determine the localization of peroxisomal¹²⁰ and mitochondrial^{121,122} mRNAs, mRNAs encoding secreted or membrane proteins to the endoplasmic reticulum (ER)¹²³, and of the *ABP140* mRNA (which encodes an AdoMet-dependent tRNA methyltransferase) to the distal pole of mother cells¹²⁴ (FIG. 4A). It has also been used to study the dynamic movement of mRNAs into processing bodies (P-bodies)^{67,125}. In addition, other bud-tip localized mRNAs have been identified using

various imaging methods^{126–128}, overall demonstrating the widespread occurrence of mRNA localization in these spherical organisms. Interestingly, *SRO7* mRNA, which encodes an effector of the Rab GTPase Sec4, localizes to at least two distinct locations under different conditions. In proliferating cells, it is transported to the bud tip in a She2–She3–Scp160-dependent manner. However, in haploid cells arrested in G1 owing to exposure to mating pheromones, *SRO7* mRNA is transported to pheromone-induced polarized membrane extensions (known as shmoo) through G-protein-coupled receptor signal transduction, which depends on the ligand-activated RBP Scp160, but not on She2–She3 (REF. 129) (FIG. 4A). Thus, the *SRO7* mRNA can be transported by different mechanisms, depending on its destination and on physiological signals.

mRNA localization in bacteria. Subcellular targeting of mRNAs was thought to occur only in eukaryotes. Electron micrographs showing polysomes attached to bacterial chromosomes¹³⁰ led to the assumption that all bacterial mRNAs are co-transcriptionally translated and that subcellular targeting was limited to proteins. This view was challenged when mRNA imaging revealed that bacterial mRNAs are localized to specific subcellular sites in a translation-independent manner¹³¹. The *lacY* mRNA, which encodes the membrane-bound lactose permease, and the bicistronic *bglG–bglF* or polycistronic *bglG–bglF–bglB* mRNAs, which encode proteins necessary for aryl- β -glucoside metabolism, localize to the cell membrane, the site of their corresponding protein products¹³¹ (FIGS 1D, 4B).

Interestingly, the signal for *bglG–bglF–bglB* mRNA localization to the membrane was found to be in the sequence encoding the first two transmembrane helices of *bglF*. This is in contrast to many eukaryotic mRNAs, which carry their zip codes in the 3'UTR. This sequence is uracil-rich, similar to various transmembrane proteins in other kingdoms¹³², which suggests the existence of an ancient membrane-targeting mechanism for mRNAs that encode transmembrane proteins. The *bglF* membrane-targeting sequence is dominant over the targeting sequences in the other genes in this polycistronic transcript, as *bglB* mRNA alone is cytoplasmic and *bglG* mRNA localizes to the poles (FIG. 4B). Deciphering the bacterial localization zip codes and their underlying mechanisms of localization is a future challenge.

mRNA localization in neurons

Morphologically and functionally distinct from unicellular organisms and somatic cells of multicellular organisms, the shape and function of the neuronal cell provide an intuitive justification for the occurrence of mRNA localization to distal subcellular regions. For example, local translation at synapses is thought to underlie persistent changes in neuronal transmission, which are crucial to learning and memory¹³³. In the past 3 decades, FISH and sequencing technologies have identified >2,000 mRNAs that are present in the dendritic and axonal portion of neurons^{9,134,135}, highlighting the vastness of the dendritic and axonal transcriptome.

Processing bodies
(P-bodies). Cytoplasmic granules that contain mRNA-degrading proteins, full-length mRNAs and mRNA fragments. Their function is unclear but is related to mRNA degradation.

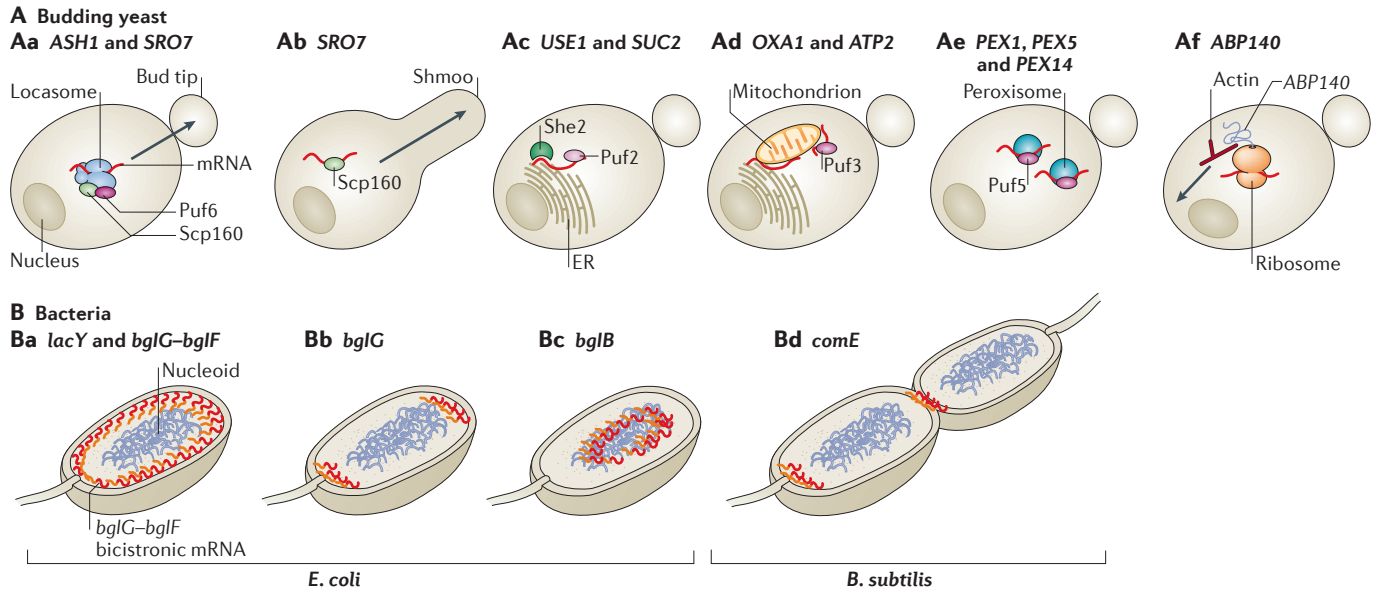


Figure 4 | mRNA localization in unicellular organisms. **A** | In budding yeast, the *ASH1* and *SRO7* mRNAs are transported to the bud tip by the locosome (which comprises Myo4, She3 and She2), Scp160 and Puf6 (part **Aa**). Following pheromone chemotaxis, *SRO7* mRNA is transported to the shmoo tip by Scp160 but not by the locosome (part **Ab**). mRNAs encoding membrane or secreted proteins (such as *USE1* and *SUC2*) are localized to the endoplasmic reticulum (ER), in a Puf2- and She2-dependent manner (part **Ac**), whereas the *OXA1* and *ATP2* mRNAs, which encode mitochondrial proteins, are targeted to mitochondria or to the mitochondrion–ER interface in a Puf3-dependent manner (part **Ad**). Some mRNAs encoding peroxisomal proteins (for example, *PEX1*, *PEX5* and *PEX14*) are localized to peroxisomes in a Puf5-dependent manner (part **Ae**). The *ABP140* mRNA, which encodes AdoMet-dependent tRNA methyltransferase, is transported to the far pole of the mother cell by direct binding of the amino terminus of its nascent protein product, Abp140, to actin filaments. The retrograde movement of actin drives *ABP140* mRNA to the far pole in a motor-independent manner (part **Af**). **B** | In bacteria, the *Escherichia coli lacY* and *bglG-bglF* mRNAs, which encode transmembrane proteins, localize to the plasma membrane (part **Ba**). *bglG* transcribed as a monocistronic mRNA localizes to the cell poles (part **Bb**), whereas *bglB* transcribed alone is cytoplasmic (part **Bc**). In *Bacillus subtilis*, the *comE* transcript, which is an operon that encodes factors for horizontal gene transfer, is localized to the nascent septum that separates daughter cells, and to cell poles¹⁷⁴ (part **Bd**).

Motored mRNA transport in neurons. Neuronal axons and dendrites, collectively known as neurites, provide long, linear tracks for studying mechanisms of motored (active) mRNA transport. Intuitively, motored transport should play an important part in mRNA movement along long neurites. Indeed, kinesin isolated from brain tissue interacts with many RBPs, as well as with the *CAMK2A* (which encodes calcium/calmodulin-dependent protein kinase type II subunit- α) and *ARC* mRNAs⁸³. More recently, kinesin was shown to interact with thousands of RNAs, which constitute about 2–5% of the total neuronal transcriptome isolated from the sea hare *Aplysia californica*¹³⁶; this is comparable to the number of mRNAs present in the extra-somatic region in the mouse hippocampus⁹, demonstrating the widespread use of active transport for mRNA localization in neurons.

Live observations of RNA movement in neurons carried out with the RNA dye SYTO14 showed that RNA material (probably mostly ribosomal) moved in a directional manner in dendrites, revealing microtubule-dependent RNA motility¹³⁷. The measured motored transport rates of 0.1 μm per second were 20 times faster than the anticipated rate of 0.5 mm per day, which was calculated from the average speed at which radioactively pulsed RNA migrated into dendrites¹³⁸. These two

measurements are in fact in agreement, as on average RNA migrates slowly into neurites, and only a subset of individual RNAs would be actively moving at the rapid speed measured in the later study. Rapid mRNA transport in conjunction with low transport probability was recently confirmed to be the case for β -actin mRNA^{53,58,62}. These comprehensive measurements of single, endogenous β -actin mRNA kinetics showed that only 10% of the mRNA molecules were actively transported at any given time with a mean speed of 1.3 μm per second⁵⁸, although the range of instantaneous rates of β -actin mRNA transport have been shown to be 0.5–5 μm per second⁵³. The motored RNA population migrated in both directions in dendrites, and the distance travelled was longer in the anterograde direction, raising the possibility that biased directionality in actively transported mRNAs leads to mRNA localization into distal regions in neurons¹³⁷. Live imaging of mRNA in neurons largely suggests that actively transported mRNAs travel further in a single trip than mRNAs in other cells^{38,39,58,60} (FIG. 5). How this behaviour is unique to neurons is likely to be a subject of future research.

The actively transported population of mRNAs in neurons has been repeatedly reported to exhibit long, processive, oscillatory movements in neurites^{58,100,137,139}.

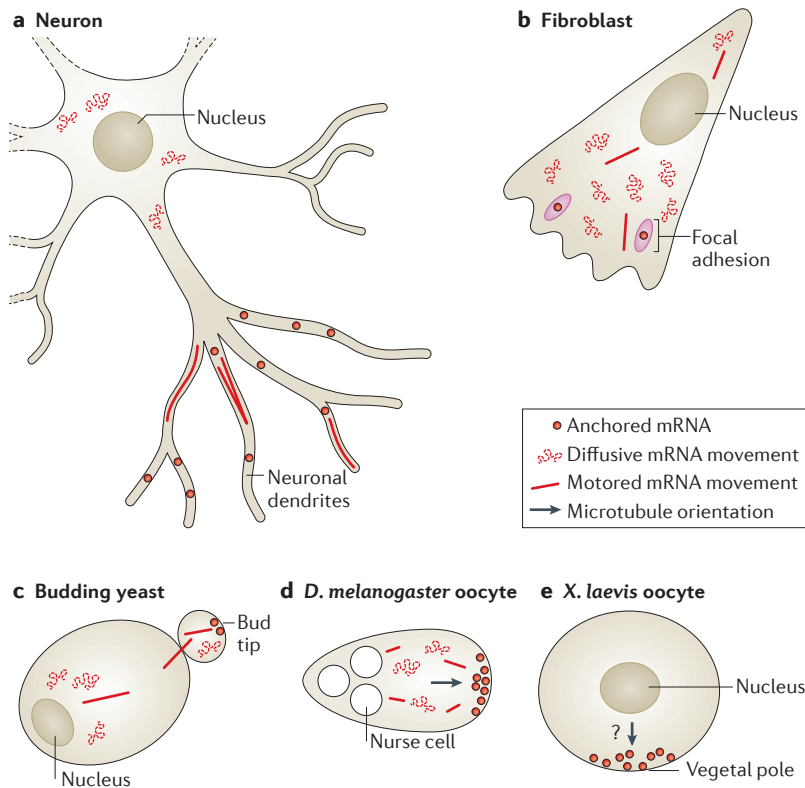


Figure 5 | Different types of mRNA movements depend on subcellular location and on cell type. **a** | Different types of mRNA movements can be observed in neurons, including diffusive movement; active, motored transport; and stalling or anchoring of mRNAs. Whereas around the nucleus, mRNAs encoding β -actin seem to move in a diffusive manner, in dendrites β -actin and ARC mRNAs seem to be largely stalled or corralled, and 10% of dendritic mRNAs are seen to undergo active transport on microtubules^{58,62,100,101}. **b** | In fibroblasts, most β -actin mRNAs are diffusing. A small percentage is transported along microtubules, and some mRNAs dwell near focal adhesions^{49,58}. **c** | In budding yeast, *ASH1* mRNAs are mostly diffusive. The localization of the *ASH1* mRNA is accomplished through myosin-mediated transport to the bud tip, where the mRNA is anchored^{38,39,175}. **d** | *oskar* mRNAs in *Drosophila melanogaster* oocytes move around mostly in a diffusive manner. A small percentage can be seen moving along the cytoskeleton for brief lengths⁵⁴. At the posterior, *oskar* mRNAs are anchored. **e** | *vg1* mRNAs in *Xenopus laevis* oocytes localize to the vegetal cortex owing to a bias in the placement of the plus ends of microtubules⁹⁴. This localization depends on two forms of kinesin, although the precise dynamics of *vg1* mRNA movement are unclear.

Synaptic plasticity
Changes in the strength of synaptic transmission in response to changes in synaptic activity, possibly during learning and memory formation.

Long-term potentiation
Long-lasting increase in the efficacy of synaptic transmission between two neurons owing to enhanced neuronal signalling or activity.

The existence of these oscillating mRNAs, which switch between anterograde and retrograde active transport, raises many questions of how such seemingly random movements result in proper mRNA localization. Single RBPs may direct this oscillatory behaviour; for example, the RBP fragile X mental retardation protein (FMRP) is associated with both kinesin and dynein in *D. melanogaster*, reiterating that a single moving mRNA particle may be simultaneously associated with different motors¹⁴⁰ (FIG. 3d). Consistent with its role in recruiting motor proteins to mRNAs^{83,140}, increasing FMRP expression in flies also results in increased processivity and frequency of actively transported mRNAs¹⁴¹. In addition to its role in mRNA transport, FMRP has a key role in translational repression of localized mRNAs in neurons⁷², similar to many RBPs that maintain dual functions to achieve local gene expression⁶⁸.

Long-term maintenance of mRNAs in neurons. Although much interest has been directed towards actively transported mRNAs, non-motile mRNAs are of interest, as they may be specifically positioned near synapses. Local translation could deliver proteins to stimulated synapses to drive or maintain synaptic plasticity¹³³. The number of synaptic sites on dendrites is likely to outnumber the abundance of single mRNAs, as shown to be the case for the abundant β -actin mRNA⁶². However, a subset of synapses seem to maintain mRNAs at the base of their dendritic spine, as shown for *ARC* mRNA¹⁰¹. Therefore, signalling cascades downstream of synaptic activity may instruct mRNAs to halt at synapses where local translation is needed (FIG. 1E). The ‘sushi belt model’ has been put forth to link observations of bidirectional active transportation of mRNA in neurites and the presence of static mRNAs at synapses¹⁴². In this model, a fraction of mRNAs is constantly in flux, moving up and down dendrites and waiting for cues to halt or be captured at a synapse.

As the contribution of local translation to synaptic plasticity is consistent with local activity, localized dendritic mRNAs may be translationally repressed much of the time. This may be accomplished through mRNA containment in neuronal RNA granules containing translationally repressed mRNAs, ribosomes and translation factors¹⁴³. smFISH of endogenous β -actin mRNA in neurons suggested that mRNAs are released from RNA granules for approximately 15 minutes following synaptic stimulation and are subsequently repackaged into them, indicating that certain mRNAs may undergo multiple rounds of translation and repression based on the activity of neighbouring synapses⁶² (FIG. 1E).

Functional importance of neuronal local translation. Although there is an abundance of evidence showing that synaptic plasticity depends on local protein synthesis¹³³, evidence on the direct role of local translation of specific mRNAs in synaptic plasticity is scarce. It is technically challenging to experimentally manipulate local translation of a specific mRNA without affecting its somatic or global translation. However, one experimental paradigm used for demonstrating the functional effects of mRNA localization is the removal of localization elements from mRNAs. The 3’UTR of *CAMK2A* mRNA mediates mRNA localization into dendrites¹⁴⁴, and removal of the 3’UTR in transgenic mice resulted in 85% loss of dendritic CAMKIIa, accompanied by deficits in long-term potentiation and memory formation¹⁴⁵. An analogous study used a conditional deletion of the axonal targeting region of the 3’UTR of importin β 1 mRNA, the encoded protein of which is known to have a role in altering gene expression during axonal injury. This deletion reduced axonal importin β 1 levels and perturbed axonal repair following injury, which is consistent with the notion that locally synthesized proteins have a role in axonal modification and repair¹⁴⁶. A third study found that the loss of localization of the mRNA encoding lamin B results in reduced membrane potential, an elongated morphology of axonal mitochondria and, ultimately, axonal degradation¹⁴⁷. These studies showed

HaloTag

A protein fusion tag derived from the enzyme DhaA from *Rhodococcus rhodochrous*. The protein can covalently bind to a synthetic chemical ligand that can be labelled with a fluorescent dye.

functional outcomes of partially or entirely removing the 3'UTR of certain mRNAs. It would be interesting to study how finer mutations or shorter deletions — specific to the mRNA localization sequences — which are now feasible using genome engineering by CRISPR–Cas9 (REF. 59), or how altering mRNA localization by artificial tethering (FIG. 2d), will alter brain functionality.

Comparing mRNA transport in spheres and trees.

How are mRNA localization regulatory mechanisms similar or different in the morphologically distinct neurons and unicellular organisms? Neuronal protrusions can be hundreds of micrometres long, whereas yeast may be only 10 µm long. Yeast cells use myosin and the actin cytoskeleton, whereas neurons mainly use kinesin, dynein and microtubules, to transport mRNAs. The reasons for this are unclear, although differences in cytoskeletal organization and motor processivity are likely to have a role. Remarkably, live imaging of different zip code-containing mRNAs in various organisms has consistently shown that 10–20% of mRNA molecules undergo active transport in neurons, *D. melanogaster* oocytes and COS cells^{54,56,58,60}, although this percentage is smaller in fibroblasts⁵⁸ (FIG. 5). An obvious difference in active transport between these systems is the run length of motored mRNAs, which is clearly regulated differently (FIG. 5). By and large, in most cells observed, mRNA localization resembles a random walk punctuated with bouts of active transport and, in some cases, ending with anchoring at a destination. Tight translational repression of mRNAs for long periods of time in neurons is reminiscent of the sequestration of maternal mRNAs in oocytes, both of which are known to be relieved by intracellular calcium influx^{56,62}. The observation that many mRNA species localize to specific organelles in yeast (FIG. 4) prompts questions of whether this also occurs in neurons or other cells. Future work will increase our knowledge of how these processes may have evolved and how they are regulated to ensure cell specificity.

Looking forward

It is becoming evident that mRNA localization has a crucial role in regulating the expression of many proteins, from bacteria to mammals. New technologies for visualizing RNA now enable subcellular, single-molecule analysis of gene expression. For example, FISSEQ¹⁴⁸ (see Supplementary information S2 (table)) of RNA can provide information on the localization of

numerous mRNAs, including splice variants and even single-nucleotide polymorphisms (for example, RNA editing products). Enhanced imaging technologies will certainly reveal more information pertaining to mRNA localization kinetics and variability.

Many questions remain regarding how mRNA localization is regulated by RBPs, and future research will reveal more complexity than we currently imagine. The full protein content of a single RNP or granule *in situ* is unknown, and there is little information on how many proteins simultaneously exert their effect on a single mRNA. Using innovative systems such as RNA purification and identification (RaPID)¹⁴⁹, which combines the utility of MS2-like systems with affinity purification (FIG. 2e), will enable biochemical analysis of specific RNP complexes, thus providing insights into their assembly on transported mRNAs. Single-molecule imaging of RNA–protein interactions *in situ* could be used to analyse RNP content, as well as subcellular localization in live cells.

Recent evidence of the colocalization of the β-actin mRNA with ribosomes in dendrites shed new light on its translation location and dynamics⁶². However, more direct methods are needed to detect mRNA translation. The FIAsh and ReAsH biarsenical dyes, which bind to tetracysteine motifs in engineered proteins, were used to identify sites of local translation in live cells¹⁵⁰. Fluorescent non-canonical amino acid tagging (FUNCAT)¹⁵¹ or single-molecule visualization of fluorescent protein production and maturation¹⁵² can be used to assess local translation dynamics. The mp-TAG system that was developed in yeast⁵¹ can provide information on the localization of the mRNA and its protein product (FIG. 2g). Organic dye labelling of proteins such as HaloTag¹⁵³ may improve imaging sensitivity and provide further information on the localization and kinetics of protein synthesis.

One key question is the functional importance of the localization of an mRNA (FIG. 1). Traditionally, mutating the zip code or RBPs involved in localization was used to study the effects of mRNA mislocalization. By using MS2-like systems to tether the mRNA to a different transport protein or to a specific location (FIG. 2d,f), it is now possible to redirect the localization of endogenous mRNAs and study the biological consequences. Combining the information on mRNA localization with that of local translation will improve our understanding of how cells are polarized and how organelles are formed.

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Competing interests statement

The authors declare no competing interests.

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