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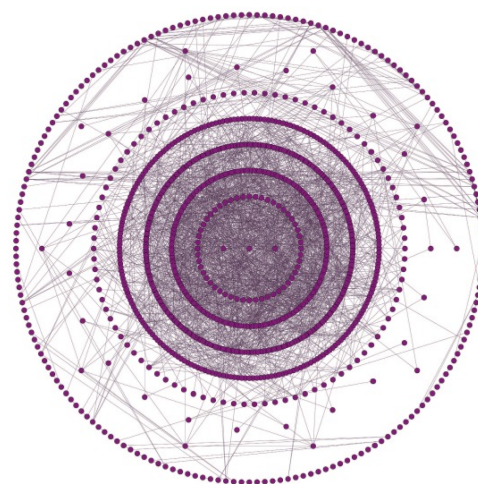

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REVIEW

Nervous translation, do you get the message? A review of mRNPs, mRNA–protein interactions and translational control within cells of the nervous system

Ross Smith · Reena Jagdish Rathod · Shalini Rajkumar · Derek Kennedy

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Abstract In neurons, translation of a message RNA can occur metres away from its transcriptional origin and in normal cells this is orchestrated with perfection. The life of an mRNA will see it pass through multiple steps of processing in the nucleus and the cytoplasm before it reaches its final destination. Processing of mRNA is determined by a myriad of RNA-binding proteins in multi-protein complexes called messenger ribonucleoproteins; however, incorrect processing and delivery of mRNA can cause several human neurological disorders. This review takes us through the life of mRNA from the nucleus to its point of translation in the cytoplasm. The review looks at the various *cis* and *trans* factors that act on the mRNA and discusses their roles in different cells of the nervous system and human disorders.

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Introduction: targeting mRNA for translation

Proteins may be directed to specific sub-cellular compartments by sequence or structural motifs contained within them, for example, the Lys-Asp-Glu-Leu (KDEL) motif that localises a subset of proteins to the endoplasmic reticulum [1]. An alternative means for bringing about selective protein localisation is to transport transcripts through the cytoplasm before anchoring and translating them at locales where the proteins they encode are needed [2–10]. The latter mechanism is used, for example, to affect localised synthesis of the calcium-calmodulin kinase α-chain (CaMKIIα) [11]. Expression of this protein is regulated in the dendrites and neuronal spines of hippocampal neurons by synaptic activity, affecting neuronal plasticity, efficiency of synaptic transmission, long-term potentiation [12] (LTP), long-term depression (LTD) and short- and long-term memory (spatial, associative and object-recognition) [13–20].

Other transported RNAs produce proteins that regulate diverse tasks, including growth cone movement (by β-actin) [21, 22], memory-linked behaviours (by the activity-related, cytoskeleton-associated protein, Arc, which brings about long-term synaptic modifications) [23], and translation (by the small, non-coding, localised BC1 RNA) [24]. In neurons, mRNA localisation is primarily confined to dendrites, although there is increasing evidence for localised mRNAs in axons [25]. A new complex found in the central nervous system (CNS) neuronal dendrites and some axons contains the Sm-like protein-1 (LSm1) protein,

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which is involved with mRNA degradation in other cell types [26]. However, cytoplasmic messenger ribonucleoprotein particles (mRNPs) containing LSm1 also include the nuclear cap-binding protein CBP80, indicating that it originally binds to transcripts in the nucleus, the cytoplasmic polyadenylation element binding factor (CPEB), LSm4, survival of motor neurons protein (SMN), and the small ribosomal subunit. This, and other evidence, supports the proposal that LSm1 and CBP80 become mRNP constituents in the nucleus. Both of these proteins move into dendritic spines following stimulation of glutaminergic receptors, suggesting that these mRNA–protein complexes, also known as mRNP contribute to regulated, localised protein synthesis and may represent stalled pre-initiation complexes, as might granules trafficking β-actin mRNA.

In this review, we chart the course of what will ultimately be localised mRNAs from their sequestration in intra-nuclear particles to their expression at the periphery of polar cells, especially neurons and oligodendrocytes.

Nuclear assembly of mRNPs

mRNA biogenesis and processing in the nucleus depends on a network of protein interactions that is required for assembly of export-competent mRNPs [27]. Formation of these complexes is interconnected with transcription, splicing and 3′ end formation. Within the nucleus there appear to be two pools of heterogeneous nuclear ribonucleoproteins (hnRNPs), one associated with nascent pre-mRNAs on the perinuclear chromatin fibres and the other with transcripts that have been released from the transcriptional machinery and are undergoing additional processing [28]. hnRNP proteins and other export factors decorate pre-mRNA co-transcriptionally and are associated with mRNPs through to their translation.

Recently, a new mechanism has been characterised, in yeast, for the co-transcriptional assembly of mRNP complexes primed for localization in the cytoplasm [29]. Nuclear association of the RNA-binding protein, She2p, with the nascent mRNA occurs co-transcriptionally through its interaction with the RNAPII-bound Spt4–5p transcriptional elongators but then recognises the nascent chain only if it contains a specific RNA element described as a ''zipcode'' (see below) [30]. She2 protein binds to a zipcode within the localising transcript [31], and directs localization of several mRNAs to the bud tip. This finding is in contrast to the typical view where mRNP assembly is regarded as a step-by-step process that depends on the successive, diffusion-driven association and dissociation of individual regulatory proteins in the cytoplasm.

Although the evidence is accumulating in favour of the nuclear assembly of the mRNP proteins, these two models are not mutually exclusive, suggesting that it is possible that major localization-determining factors are assembled in the nucleus but that other proteins are added later, or exchanged at different stages of the mRNP transport or translation. However, this example has been characterised in yeast and it remains to be seen if it is mimicked in neurons, but it should be noted that several of the proteins involved in mRNP assembly, transport and translation are evolutionarily conserved. Interestingly, zipcode binding proteins 1 and 2 (ZBP1 and ZBP2) are the proteins that recognise the zipcode in β-actin mRNA and are responsible for the localization of this mRNA in the growth cones of neurons [32]. ZBP1, ZBP2, and β-actin mRNA were found colocalised in nuclear foci identified as active β-actin transcription sites [33, 34], suggesting that they assemble on mRNA co-transcriptionally to create mRNPs that have a pre-defined destination for translation of their cargo, thereby mimicking the mechanisms described in yeast.

Nucleocytoplasmic RNA trafficking

Originally it was thought that mRNA-binding proteins within mRNPs were exchanged for hnRNP proteins at or near nuclear pores, but it is now established that proteins such as hnRNPs A, B and C shuttle between the nucleus and cytoplasm (reviewed in [35]), suggesting that hnRNP proteins and mRNA are exported to the cytoplasm together as a complex. Interestingly, the recruitment of shuttling hnRNPs to nascent transcripts for export from the nucleus appears to be transcription dependent [36]. Different classes of RNA (e.g. tRNA or mRNA) are exported as ribonucleoprotein complexes via different pathways in which distinctive *cis*-elements are recognised on the RNA or on the RNA-bound protein.

Nascent pre-mRNAs form complexes with hnRNP proteins that undergo substantial remodelling during mRNA maturation and export [37]. Mili and co-workers isolated three mRNP and pre-mRNA complexes, which included the hnRNP A1 protein and postulated that these three complexes constituted steps along the nucleocytoplasmic pathway. hnRNP A1 was found in two distinct structures in the nuclei of HeLa cells: one contained pre-mRNA and mRNA and the second contained several other shuttling members of the hnRNP protein family, but not pre-mRNA.

How mRNAs travel from sites of transcription to the nuclear pore complex (NPC) where they associate with karyopherins prior to transfer from the nucleus to the cytoplasm is not well understood, though it has been established that there is an energy-dependent component in this motion. Nor has it been resolved how directionality in mRNA export is brought about, or how nuclear export factors are dissociated from the mRNPs after passage through

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the NPC. Several mechanisms have been proposed, including involvement of the RNA-binding protein, Y14 protein, which is part of the exon junction complex (EJC) [38, 39] and part of a multi-protein complex that also contains the mRNA nuclear export factor 1 (NXF1, also known as transporter associated with antigen processing, TAP), which is discussed in greater detail below.

Numerous factors are involved in mRNP translocation through the NPC to the cytoplasm, where the particle is reshaped by release of some proteins and reuptake into the nucleus of others [40]. A subset of hnRNPs, including hnRNP A1, remains bound to the exported RNA and cycles between the nucleus and cytoplasm, with nuclear localisation signals (NLSs) fostering reuptake into the nucleus. However, it is uncertain whether these hnRNPs have an active role in mRNA export. TAP is the main export receptor for general cellular poly(A)$^+$ mRNA [41], a member of a family of putative nuclear export receptors that have the main features of importin-β but share little sequence identity with it. The conserved TAP/p15 heterodimeric transport receptor may drive the translocation of mRNPs through the nuclear pores via recruitment to NPCs and sequential interactions with the FG (Phe-Gly) repeats of the nucleoporins [36], but it lacks the Ran-binding domain found in all karyopherins.

Staufen2 (Stau2) is another nuclear shuttling protein [42]. Two distinct pathways for Stau2 nuclear export have been elucidated: one is exportin-5 (Exp5)-dependent whereas the other is a Chromosome Region Maintenance 1 protein (CRM1, also known as exportin 1)-dependent pathway [43]. Both Exp5 and CRM1 are importin-β family members (Fig. 1). Exp5 binds RNA directly by recognising mini-helix-containing RNAs, and in conjunction with a GTP-bound form of RanGTPase assists the export of RNA molecules [44], whereas CRM1 binds to the nuclear export signal (NES) of Staufen to mediate nuclear export [45]. This suggests that Stau2 acts as an adaptor protein for nuclear export of RNA in the Exp5-dependent pathway: it may additionally become a part of the mRNP complex in the nucleus and assist its nucleo-cytoplasmic shuttling. This implies that, before nuclear export, Stau2 moves to the nucleolus and is assembled into mature RNPs [46, 47]. The TAP protein complex is known to mediate the bulk of mRNA export, not CRM1 [47, 48], however, CRM1-dependent nuclear export of some cellular mRNAs does occur [47, 49, 50]. Interestingly, Stau2 interacts with nuclear pore protein p62, TAP, and the Y14-Mago heterodimer, providing a plausible hypothesis that Stau2 links nuclear RNA processing and cytoplasmic *trans*-localization in neurons [51].

RNA-binding proteins are needed as a bridge for the interaction between TAP and mRNA [27]. Over-expression of TAP stimulates the export of mRNAs that are otherwise retained in the nucleus. It has been shown that it binds to the constitutive transport element (CTE) in retroviral RNAs and promotes export. However, in normal mammalian pathways TAP is likely to bind to mRNPs through protein–protein interactions rather than protein–RNA interactions. Finally, nuclear export is facilitated by the karyopherin receptor, which recognises proteins with a nuclear export signal (NES) and shuttles them to the cytoplasm.

Nucleoporins interact with two binding sites on TAP, mediating interactions between export cargo and NPC components that are needed for translocation. The roles of

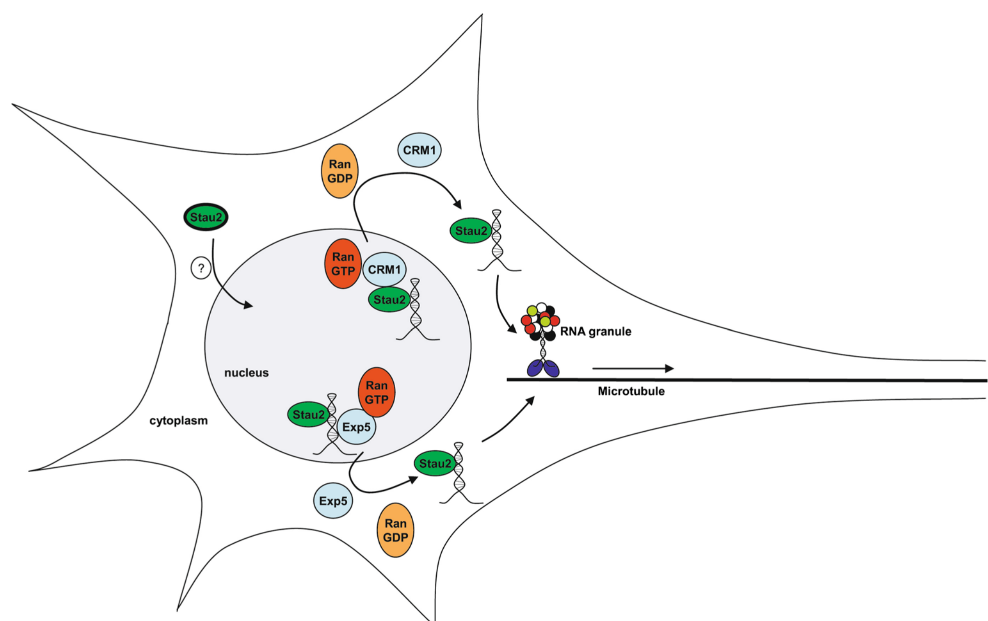

Fig. 1 Stau2 mediated nucleocytoplasmic shuttling. A schematic representation of a neuron showing the two proposed mechanisms in which Stau2 may be invovled in export of RNA from the nucleus to the cytoplasm. In the Exp5-dependent pathway, an RNA containing a minihelix acts as an adapter to link Stau2 and Exp5 and the complex is exported. In the Crm1-mediated pathway a separate pool of RNA, that do not bind Exp5, are bound by Stau2 and exported to the cytoplasm. Both pathways use the hydrolysis of GTP by Ran for export, subsequently Stau2-RNA complex is incorporated into an RNA granule for transport along the dendrite (adapted from [47])

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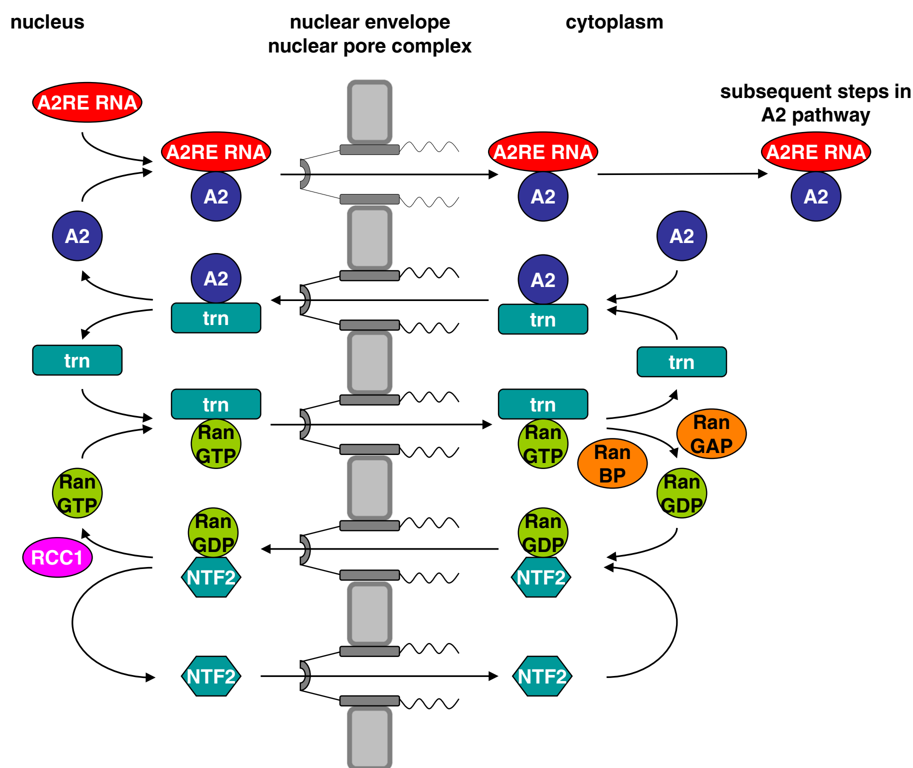

Fig. 2 A2RE mediated nucleo-cytoplasmic shuttling. A2RE-containing RNA and its cognate *trans*-acting factor, hnRNP A2, are exported from the nucleus to the cytoplasm as a complex. The latter cycles between the cytoplasm and the nucleus. The carriers transportin 1 (trn) and NTF2 shuffle cargo or Ran molecules through the nuclear pore. The ratio of RanGTP to RanGDP determines the direction of cargo movement. RCC1, the regulator of chromosome condensation 1, binds to the nuclear GTP-binding protein, Ran. This figure is reproduced here with the kind permission of Prof. John Carson, Prof. Elisa Barbarese, and Portland Press

transportin 1, Ran/GTP Ran/GDP, RanGAP (Ran GTPase activating protein), RanBP (Ran binding protein) and the nuclear transport factor 2 protein (NTF2) in nucleocytoplasmic transport of hnRNP A2 and its target mRNAs which contain a defined zipcode (GCCAAGGAGC), the hnRNP A2 response element (A2RE), are depicted in Fig. 2 [52]. These proteins make up a transport system that recognises export motifs in cargo (e.g. the NLS and NES sequences in hnRNPs A/B) that is to be exported from the nucleus.

Coupling of RNA splicing and nuclear export

Splicing is required for rapid and efficient nuclear export of mRNA. Pre-mRNA splicing and export are coupled: small clusters of proteins, the EJCs, bind to mRNA because of splicing and may mark the mRNA for export from the nucleus. Conversely, nuclear retention of hnRNP A1 occurs when it is associated with pre-existing splicing complexes, which may serve as a means for retaining unprocessed transcripts in the nucleus.

TAP preferentially binds spliced mRNA in vitro and in vivo and in concert with its p15 cofactor, interacts with both adapter proteins, including REF (RNA export factor) binding proteins, and components of the NPC to affect transport through the nuclear pores [53]. Two members of the serine/arginine-rich (SR) family of splicing factors, 9G8, alternative splicing factor 1 (ASF) and pre-mRNA-splicing factor 2 (SF2), also act as adapters, interacting with TAP [53]. Dephosphorylation of the SR proteins, which are normally phosphorylated near the C-terminus, enhances their binding to TAP both in vitro and in vivo [54], thus augmenting the chain of communication linking splicing of pre-mRNA to its export to the cytoplasm as mRNA.

Cytoplasmic granule assembly and composition

Granules are large aggregates formed in the cytoplasm from RNAs and RNA-binding proteins following mRNA export from the nucleus. Each granule may contain several different mRNAs with the same *cis*-acting elements, but they do not transport RNAs with different, or mutant, *cis*-acting elements. Furthermore, the granules will incorporate multiple copies of one of the cognate hnRNP proteins (*trans*-acting factors) for the trafficking. Granules participating in, for example, the hnRNP A2 response element (A2RE) driven cytoplasmic trafficking can accommodate multiple mRNA molecules [55] and different *trans*-acting factors [56]. hnRNP A2 and A3 proteins, and RNAs bearing the A2RE *cis* element, segregate into such cytoplasmic granules and may therefore also participate in cytoplasmic transport. In hippocampal neurons, many granules appear

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to contain only one of these two hnRNP paralogs; few have both proteins, suggesting that they may have separate and specific roles in the transport or sorting of their target mRNAs [3].

Several groups have isolated different populations of cytoplasmic trafficking granules [17, 57] for proteomic analysis: as discussed below, these studies have yielded disparate results. Kanai and co-workers used antibodies to conventional kinesin to immunoprecipitate-fractionated granules that contained this motor protein (reviewed in [58]). The mRNAs of Activity-regulated cytoskeleton-associated protein (Arc also known as Arg3.1) and Ca^{2+}/calmodulin-dependent protein kinase II alpha (CaMKIIα) and 42 proteins colocalised with these kinesin-associated granules in dendrites and many of them were found to be important for the localisation of a CaMKIIα reporter, including hnRNPs A1, A2, D, A0, U, Purα, Purβ, Staufen1, polypyrimidine tract binding protein-associated splicing factor (PSF), elongation factor EF-1α, and three FMRPs (Fragile X mental retardation proteins FMRP, FXR1 and FXR2). Many of these components are discussed in further detail below. These granules appeared to lack the eukaryotic initiation factors 4E and 4G (eIF4E, eIF4G) and tRNAs and were therefore assumed not to be translationally active [59], in accord with similar studies mentioned below [60].

A2RE-containing granules have a scaffold of TOG (tumour over-expressed gene), which is an essential component of the granules and binds multiple copies of hnRNP A2 [61]. Each of these protein complexes associates with a cognate A2RE-containing mRNA that encodes one of the proteins FMRP CaMKIIα, Arc, NG (neurogranin), PKMζ [brain-specific protein kinase C (PKC) isoform protein kinase M-ζ] and the microtubule-associated proteins (MAP2 and MAP1B). FMRP plays a central role in switching these granules from a state in which the RNAs are quiescent, to being translationally activate. Phosphorylation of FMRP results in suppression of translation of the mRNAs encoding itself and the other proteins which become components of the postsynaptic density in response to synaptic activation. The granules additionally possess other components required for translation, including the shank proteins which are also scaffolding components of the postsynaptic density (PSD). Transport of shank1 mRNA in neuronal dendrites of the hippocampus and cerebellum is mediated by a dendritic targeting element in its 3′-untranslated region (UTR) [62]. Shank family members (shank1–3) connect different types of glutamate receptors in the PSD of excitatory synapses. Falley et al. [62] showed that the transported mRNP cargo contained eIF2α, DEAD (Asp-Glu-Ala-Asp) box helicase 1 (DDX1), staufen1 and FMRP proteins and that the cargo is transported via the Kinesin heavy chain isoform 5C (KIF5C) motor protein. Interestingly, translation is strongly inhibited by a GC-rich 5′-UTR; in addition, internal ribosomal entry sites previously detected in other dendritic transcripts are absent in the shank1 mRNA. A concept emerges, in which dendritic transport of different mRNAs may occur collectively in a single RNP complex via a staufen1- and KIF5-dependent pathway, whereas their local translation is controlled individually by unique *cis*-acting elements.

Some proteins may have a more general function in granule assembly or trafficking, rather than being *trans*-acting trafficking factors. The dsRNA-binding protein, Stau1, is one such protein: it appears to assist granule formation and is involved in targeting of dendritic RNAs. As noted in the previous paragraph, it co-precipitates with numerous other proteins, many of which may be *trans*-acting factors that bind different targeting elements [2] and may act as a general assembly platform for granules. Stau2, a paralog of Stau1, is found with Stau1 in the somatodendritic compartment where both proteins colocalise with microtubules [42]. There is no evidence that Stau2 binds directly with a *cis*-acting sequence but, like the A/B hnRNPs, Stau1 and Stau2 segregate to different populations of granules. As discussed above, following export from the nucleus RNP particles undergo a remodelling in which A2RE-containing RNAs are recruited to the granules ready for dendritic transport. Granule assembly may be directed by the partitioning of *trans*-acting factors between the nucleus and cytoplasm, assembling in the cytoplasm of neurons, oligodendrocyte progenitors, and oligodendrocytes, where the hnRNP A2 concentration is ~20-fold lower than in the nucleus and aggregation of A2RE into oligomers is favoured [28, 52].

Microtubule-associated, mRNA-containing granules contain many components of the translation apparatus. Mouse oligodendrocyte granules include aminoacyl-tRNA synthetases, elongation factors, ribosomal RNA, mRNAs [60], and the motor proteins kinesin and dynein [63]. A wide range of additional components are found in granules from embryonic rat brain and cultured hippocampal neurons. The RNA-binding proteins, ras-GTPase SH3-domain binding proteins 1 and 2 (G3BP1, G3BP2), the mammalian embryonic lethal abnormal visual (ELAV)-like neuronal proteins (HuB, HuC and HuD) and many of the hnRNPs, including A1, A2/B1, and E2, have been identified by proteomics of purified trafficking granules [57]. It is interesting to note that viruses can hijack these components to facilitate the transport of their own RNAs [55, 64]. The granules from embryonic rat brain are highly enriched in ribosomes whereas adult rat brains are reported to lack these components, reflecting the greater need for generalised translation to bring about normal maturation. Despite the large number of proteins identified in the granules, few of these proteins have been verified as components of transport granules. One exception is the HuD protein, a component of

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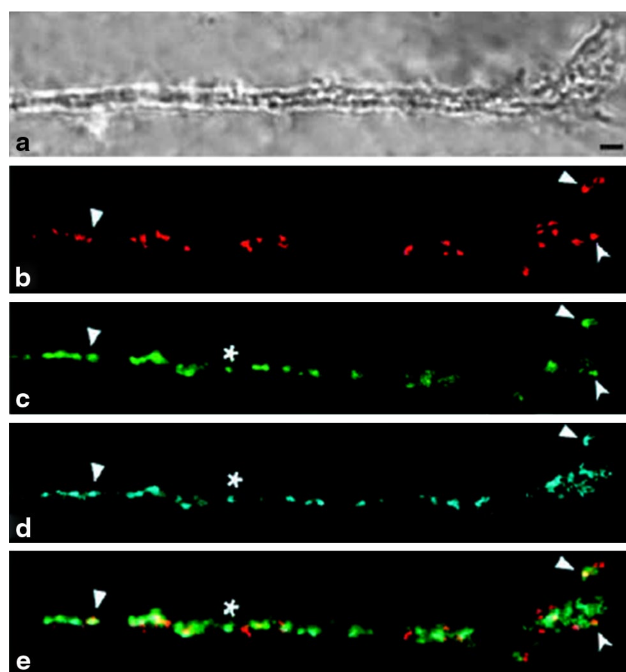

Fig. 3 HuD, KIF3A and tau mRNA colocalise in RNP granules. **a** Phase image: the growth cone is shown on the *right side* of the *panel*. Colocalisation of tau mRNA (**b**, *red*), HuD protein (**c**, *green*) and KIF3A (**d**, *cyan*) to yield the merged image presented in e of the axon and growth cone of differentiated P19 cells. The *curved arrowhead* denotes colocalisation of the three components (*white*). The *asterisk* denotes colocalisation of HuD and KIF (*light green*). The *straight arrowhead* denotes colocalisation of HuD and tau mRNA (*yellow*). *Bar* 1 mm (′40,000 magnification). Reproduced with permission of the Company of Biologists from [65]

microtubule-associated *tau* RNP granules that are localised to axons along with the kinesin KIF-3, resulting in granule attachment to, and movement along, the microtubule (MT) tracks (Fig. 3) [65]. These results suggest that HuD can serve as a link to the axonal microtubules (mRNA transport in *Drosophila* was recently described elsewhere [66]). This is consistent with previous studies that have suggested a role for HuD in mRNA granule assembly and their shuttling from the nucleus to the cytoplasm [67, 68].

RNA granules isolated from neurons are highly enriched in Staufen and ribosomes, but they are generally translationally silent. However, upon KCl-induced depolarisation of neurons many granule mRNAs move to polysomes, resulting in a less compact ribosomal organisation [59]. The local release of mRNAs and ribosomes from granules may serve as a mechanism for linking RNA localisation to translation, but this would not be selective for a subset of mRNAs. Furthermore, the general assembly and disassembly of granules is another way of regulating temporal translation of specific mRNAs, such as Cyclin B1 [69].

Multiplexing in trafficking granules

RNAs with the same trafficking pathways are assembled into the same population of granules in contrast to RNAs with destinations to different pathways (i.e. different *cis*-acting elements and *trans*-acting factors), which segregate into different granule populations [70]. Thus, myelin basic protein (MBP), green fluorescence protein (GFP) and Connexin 32 (Cx32) RNAs assemble into granules that are destined for the myelin compartment of oligodendrocytes, the perikaryon, (cell body of the neuron not including outgrowths such as axonal or dendritic processes), and the endoplasmic reticulum, respectively. Cx32 RNA, which does not contain an identifiable A2RE-like trafficking sequence, is not assembled into the same granules as hnRNP A2–bound RNAs, but is found in granules that contain hnRNP A2, indicating hnRNP A2 has both a general role in granules and a distinct role for the triage of mRNAs containing a *cis*-acting element that it directly binds to. Granules containing multiple different mRNAs could be expressed co-ordinately, but the evidence favours the view that the A2RE-containing RNAs are translated one or two at a time, because there are typically only very few polyribosomes in the dendritic spines of neurons or locations of myelin membrane synthesis in oligodendrocytes [8, 71].

There are up to ~30 RNA molecules in single mammalian trafficking granules but the absolute number and species of RNA are variable, with granules containing different species of RNA that contain the same cytoplasmic transport motifs, This implies that the granules can multiplex (reviewed in [8]). A2RE-like motifs are found in a multitude of RNA molecules including mRNAs for ARC, CaMKIIα, neurogranin, MAP2, MAP 1B, PKMζ and FMRP: the latter using 5′ CGG repeats rather than the more canonical A2RE-like sequence in the 3′ UTR or coding region. Upon activation, *Arc* and *CaMKIIα* expression is increased and these mRNAs are relocated from the perikaryon to the dendrites and dendritic spines. Both mRNAs localise at synapses that have been activated and the translated proteins are assembled into the synaptic junctional complex [23]. This is important for co-expression of these two RNAs in maintenance of long-term plasticity [55]. Many of the proteins encoded by these synaptically localised RNAs are themselves associated with synapses and respond to activation of NMDA receptors.

hnRNP A2 binds A2RE-containing RNAs, capturing them in granules. However, it is uncertain which molecules the A2RE-bound hnRNP A2 is associated with: for example, its interaction with the motor protein kinesin may be indirect, implying that other factors dictate which hnRNP A2 molecules are trafficked and which are not. This may explain how hnRNP A2 binds to some A2RE-free RNAs

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e.g. Cx32, but this interaction does not lead to subsequent trafficking of this RNA.

cis-Acting elements: sequence and structure

Many neuronal *cis*-acting elements have been described. Some of these elements may be defined by their nucleotide sequence, whereas others appear to be dependent on the three-dimensional structure of the RNA. Some are small and well defined (in segments of 6–30 nt), whereas others can only be loosely placed within regions of several hundred nucleotides. But, of course, the atoms within the RNA that make contact with the *trans*-acting factor may include many nucleotides from widespread locations in the nucleotide sequence. From known three-dimensional structures of RNA–protein complexes it is evident that elements such as the 11-nucleotide A2RE11 or the 14 nt Y element, which are described below, are of sufficient size to fully occupy the binding site on their cognate proteins.

β-Actin zipcode element

The β-actin zipcode, one of the best studied, has been identified as a 54-nt sequence directing β-actin RNA to growth cones of developing neurons, which is important for establishing cell polarity and neurite outgrowth [72, 73]. The nucleotides within this zipcode that make contact with the bound protein have not been determined, but it has been proposed that the 3D structure of the RNA zipcode is important. Even if the trafficking motif has no fixed 3-dimensional structure in the isolated RNA, it may well adopt a more persistent conformation when bound to protein. ZBP1 [which has four hnRNP K homology (KH) domains and two RNA-recognition motifs (RRM), and is primarily cytoplasmic] was initially shown to bind the β-actin zipcode. Subsequently ZBP2, a homolog of KH-type splicing regulatory protein(KSRP),which is involved in alternative pre-mRNA splicing, was found to bind first to the nascent β-actin mRNA. After nuclear export, ZBP1 attaches to the trafficking granules and remains with them until the mRNA is translated at the cell periphery [34]. hnRNP R also interacts with the 3′ UTR of β-actin mRNA [74] and is implicated in the axonal transport of β-actin mRNA in zebrafish and in mouse motor neurons. Suppression of hnRNP-R in developing zebrafish embryos resulted in reduced axon growth in spinal motor neurons. shRNA-mediated knockdown in isolated embryonic mouse motor neurons reduced β-actin mRNA translocation to the axonal growth cone, which is paralleled by reduced axon elongation [75].

Localisation of ZBP1 granules in dendrites and actin-rich protrusions (i.e. in both filopodia and spines of rat hippocampal neurons) regulates the density of filopodial protrusions and synapses [76]. Within neuronal growth cones, β-actin mRNA appears to be associated with microtubules, and actin protein is enriched in structures undergoing polymerization [21]. These results reveal a novel function for the zipcode mRNA localization element and its binding protein in the regulation of dendritic morphology and synaptic growth in cultured rat hippocampal neurons [77].

The structure of the A2RE *cis*-acting element

The RNA transport sequence (RTS) for mRNA (also referred to as the A2RE) or smaller segments of this sequence (A2RE11: GCCAAGGAGCC) are necessary and sufficient for trafficking of myelin basic protein RNA [78]. This *cis*-acting element was subsequently found in many mRNAs that are transported in oligodendrocytes and neurons. Identification of these elements is not always straightforward, as evidenced by the differing views that have been held about the location of the A2RE motif within the RNA encoding CaMKIIα [79–81]. By contrast with the other A2RE-like sequences, the *cis*-acting element in FMRP is a highly repeated CGG trinucleotide, which may reflect G-quadruplex structures seen in other mRNAs [82]. Even the A2RE, one of the shortest *cis*-acting elements identified, has been postulated to form a 3-dimensional kink (K)-turn structure that is recognised by RRM-containing proteins [83]. These turns, which were discovered in the three-dimensional structure of the bacterial ribosome, are mostly involved in protein binding: the K-turn is a protein recognition element [84]. They have stems of duplex RNA and an internal loop [83] but, as noted in previous studies [85, 86], even the short, single-stranded A2RE11 may adopt a kink-turn conformation when bound to hnRNP A2. Their cognate *trans*-acting factors of course display the same rich variety as these *cis*-acting elements.

A snapshop of other *cis*-acting elements

Other *trans*-acting factors also bind short oligonucleotides. MBP mRNA of oligodendrocytes, and CaMKIIα RNA in neurons, may contain different *cis*-acting elements that impart different functions (e.g. cytoplasmic polyadenylation element, CPE [87]). CPE-binding protein (CPEB) may act as a translation-masking (repression) protein and as a polyadenylation-inducing factor. CPE sequences are variable but there is a consensus sequence, UUUUUAU, recognised by CPEB and there may be multiple CPEs in a single mRNA. The RNA-binding protein HuR affects the stability of specific short-lived mRNAs that have an ARE (AU-rich element, with an AUUUA core sequence) in their 3′ UTR. Translin (testis-brain RNA-binding protein, TB-RBP) is

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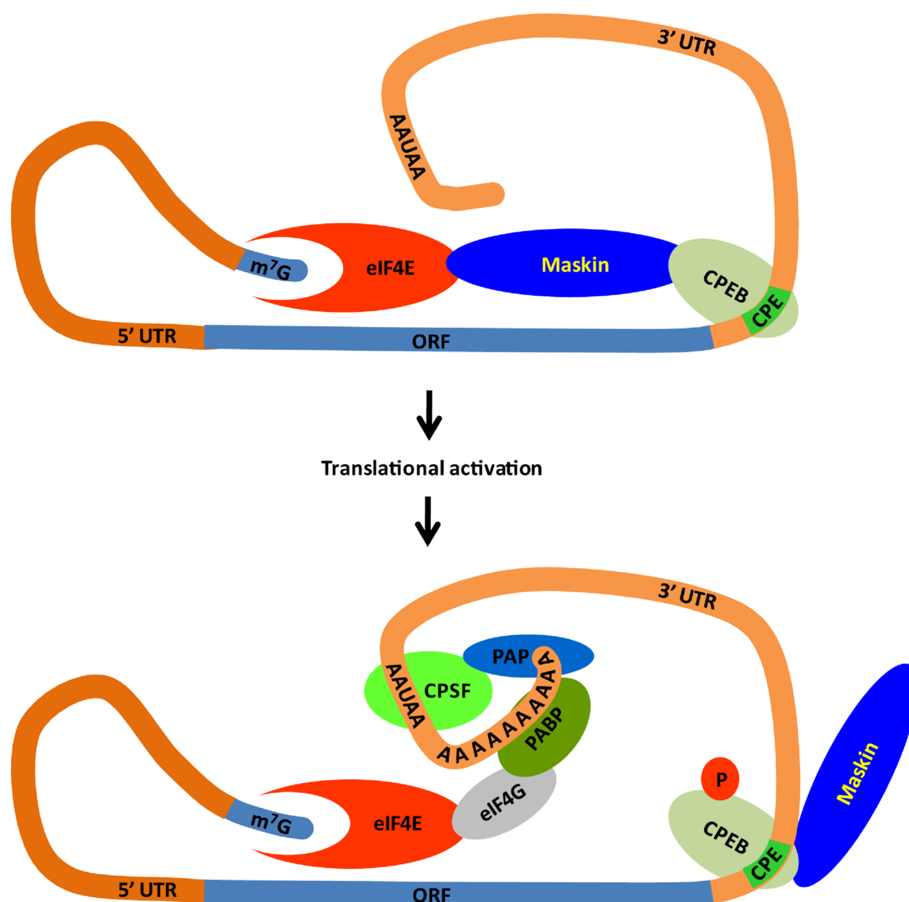

Fig. 4 Derepression of Translation. Repression of translation is shown in the *upper panel* in which unphosphorylated cytoplasmic polyadenylation element-binding protein (CPEB) is depicted in a complex bound to its recognition sequence (CPE) and maskin. In this configuration eukaryotic translation initiation factor 4E (eIF4E),which interacts with the 5′ 7-methyl-GTP cap (m^7G) is unable to interact with eIF4G. Upon stimulation, CPEB is phosphorylated (*lower panel*). Derepression of translation occurs when phosphorylated CPEB interacts with cytoplasmic polyadenylation specificity factor (CPSF) which allows poly(A) polymerase (PAP) to extend the polyadenylated tail and recruits poly(A) binding protein (PABP). These changes allow PABP to bind eIF4G through the release of the eIF4E-Masking interaction allowing translation of the open reading frame (ORF). Adapted from [180]

another protein that recognises a small oligonucleotide, the Y-element CTGAGCCCTGAGCT. Blocking translin binding to this element disrupts the localization of the mRNAs encoding protamine 2, ligatin and CaMKIIα [88]. Longer *cis*-elements include the recently identified a 66 nt element in the 3′ UTR of sensorin mRNA regulating its cytoplasmic transport to the synapse [56].

Although some defined *cis*-element sequences have been identified, precise RNA recognition sequences are not always apparent. For example, Staufen and other proteins known to bind to mRNA in mRNP complexes, recognise three-dimensional structures within mRNA in a non-sequence-specific manner [89]. It appears that a mixture of sequence-specific and structure-specific motifs is involved in defining regulated mRNA transport.

Trafficking: *trans*-acting factors

RNA-binding proteins are mosaic proteins that draw on a limited repertoire of domains to generate multiple structures and functions. Some of the more common domains include single and tandem RNA-recognition motifs (RRMs; also described as RNA binding domains, RBD), K homology (KH) domains, glycine-arginine richboxes (RGG), Gly-rich regions, Arg-rich domains, and OG (oligonucleotide/oligosaccharide-binding) domains. Several neuronal proteins have been shown to bind trafficking elements within the 3′ UTRs of localised mRNAs, including ZBP1 in fibroblasts [90, 91] and neurons [92], and ZBP2 [93] binding to the 54 nt β-actin zipcode, hnRNP A2 binding to the 21 nt RNA cytoplasmic transport sequence of MBP mRNA [94], MAP2 RNA *trans*-acting factor (MARTA) binding to the dendritic targeting element (DTE) of MAP2 mRNA [95] (MARTA1 is present in cytosolic, ribosomal salt wash (RSW), and nuclear fractions, but MARTA2 is preferentially enriched in the RSW [95]), and finally CPEB, which binds the cytoplasmic polyadenylation element, modulating translation and mRNA localisation [87, 96] (see also Fig. 4).

Many *trans*-acting factors are expressed as isoforms that have different sub-cellular locations. For example, CPEB is expressed in several alternatively spliced isoforms that have specific tissue locations and functions [97]. ZBP1 is found in neurites of young neurons whereas the K homology domain protein ZBP2 is most abundant in the nucleus, but shuttles rapidly between nucleus and cytoplasm and mediates binding of ZBP1 to nascent β-actin mRNA during

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transcription [34]. Finally, FMRP is also expressed as multiple isoforms that modulate short- and long-term memory [20].

Four isoforms of human hnRNP A2/B1 are produced by alternative splicing of exons 2 and 9. These isoforms are localised to different parts of the cell: the major isoforms (A2 and B1) are almost exclusively retained in the cell nucleus whilst the less abundant isoforms (A2b and B1b) are found at low levels in the nucleus but also in the dendrites and spines of neurons, and throughout the processes in oligodendrocytes [98].

Neurotropin-3 (NT-3) regulates local protein synthesis by stimulating localisation of β-actin mRNA [22] within growth cones and filopodia during neuronal development. This localisation is also stimulated by forskolin and dibutyrate-cAMP. In addition to stimulating β-actin mRNA and protein localisation, NT-3 also promotes actin polymerisation.

A number of these factors including (rat) MARTA1, ZBP2 (DTE-binding) and (human) KH-type splicing regulatory protein (KSRP) are orthologs and consequently share similar domain structures. For example, rMARTA1 and hKSRPare 98 % identical in amino acid sequence [95]. Similarly, Purα and Purβ share 71 % sequence identity, and these regions contain highly conserved basic 23-residue class I repeats and acidic 26-residue class II repeats [99].

HuD, like a number of other proteins, displays activity-dependent expression in neurons and dendritic localisation following KCl-induced stimulation of hippocampal neurons. Immunoprecipitation showed HuD associates with a number of mRNAs including those encoding neuritin, CaMKIIα, and GAP-43. HuD-associated mRNAs colocalise with poly(A) binding protein (PABP) and cap-binding eIF4E, suggesting translational regulation of the HuD-associated transcripts. This molecular mechanism also involves the *N*-methyl-D-aspartate (NMDA) receptor, which affects the expression of HuD [77]. The Hu family of *trans*-acting factors recognises AU-rich elements. Another hnRNP, CBF-A, which is closely related to hnRNP A/B [100], also binds the A2RE and is found along with hnRNP A2 in trafficking granules: it appears to be a *trans*-acting factor that directs the trafficking of myelin basic protein mRNA [101, 102].

FMRP has two RNA-binding KH domains and an RGG domain that binds to intramolecular G-quartets in the 3′ UTR of the target mRNA [103–105]. The RGG box of FMRP protein is flexible and highly charged, therefore an induced fit between the RGG box and the G-quartets may mediate binding of a large number of different mRNA targets to this protein. A possible explanation for sorting and specificity among potential RNA targets may come from the finding that both RNA context and cation composition dictate a large complexity of conformations [105]. In the MAP1B-RNA interaction with FMRP [105], G-quartet formation is mediated by RNA dimerization and oligomerization. These findings suggest that intermolecular RNA:RNA interactions could be involved in the process of mRNP formation by regulating the number of particles of a specific mRNA present in a given complex [105]. Besides G-quartets, FMRP has also been shown to interact with RNA aptamers presenting loop–loop pseudo-knot-specific motifs or the “kissing complex” [106]. The kissing complex is a basic type of RNA tertiary contact and is created by the base pairing of complementary sequences in the apical loops of two hairpins. Intramolecular kissing complexes have been found in many RNA structures, ranging from 75-nt tRNAs to megadalton ribosomes; these complexes are also critical for many biological processes, such as dimerization of retroviral genomic RNAs (reviewed in [107]). FMRP is discussed more fully in the section below in relation to its role in disease.

Cytoplasmic trafficking

RNAs destined to be localised within neurons begin their cytoplasmic journey after they have been incorporated into trafficking granules. Interestingly, the signal for transport is carried on the copies of mRNA within the granule. In the absence of this RNA-borne trigger the RNA may still be incorporated into granules, but they are not competent for trafficking. However, the RNA is not directly responsible for localization, instead it is responsible for the activation of spatially restricted translation that results in a corresponding localization of the protein encoded by this RNA.

RNA granules lacking a *cis*-acting localisation element are found in the perikaryon, suggesting that they are associated with the minus ends of microtubules and are retained there by active dynein. By contrast, active kinesin transports the granules to the plus ends of microtubules at the cell periphery. The final location adopted depends on the presence or absence of *trans*-acting factors such as hnRNP A2, which regulates the balance of these two motor proteins and favours the use of kinesin. Translocation of granules containing A2RE RNAs and hnRNP A2 is biassed towards the plus ends of microtubules, i.e. to the periphery of oligodendrocytes and neurons [4], although there is rapid movement in both directions. CaMKIIα mRNA-containing granules in neurons and myelin basic protein mRNA-containing granules in oligodendrocytes [108] have also been observed to undergo bidirectional motion [8, 58, 109], with the net movement determined by the duty-cycle of the molecular motors. Bidirectional transport of these granules is maintained by the plus-end-directed conventional kinesin (KIF5) and minus-end-directed dynein molecular motors [58, 63, 70, 110]. Individual transported

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mRNAs in living mammalian cells move rapidly and directionally on microtubules, moving large distances in both directions. The presence of the β-actin zipcode increases both the frequency and length of these movements [111], which involve both microtubules and microfilaments. The importance of hnRNP A2 for granule formation has been illustrated recently by co-injection of neurons with fluorescently labelled RNA and antibodies directed at exon 9 (a control) or at the junction of exons 8 and 10. The latter antibody binds hnRNA A2b (and possibly B1b), sequestering it from its usual role in RNA trafficking [98]. In a similar approach, Tübing et al. [112] coinjected and simultaneously visualised, in live hippocampal neurons, MAP2 and CaMKIIα RNAs that had been labelled with different Alexa fluorophores. These RNAs were distributed to the neuronal dendrites. The equivalent endogenous RNAs were detected using florescent in situ hybridisation (FISH) with DIG- and fluorescein-labelled probes. The localization of these RNAs mirrored the patterns of the corresponding endogenous RNAs, revealing that neuronal transcripts are differentially sorted in dendritic granules. Some RNAs are sorted to the same particles, whilst others are transported in different granules. The latter behaviour has been shown graphically for hnRNPs A2 and A3 [113], and for MAP2 and CaMKIIα [112].

Repression and reactivation of translation

Although transcription is frequently the step at which the cell exercises control over gene expression there is a rapidly expanding pool of identified genes for which primary control is exerted at the level of translation, commonly at the initiation step. As discussed above, a number of localised mRNAs appear to be translated only following their arrival and anchoring at their subcellular destination. With a few exceptions, the triggers for imposing and lifting the translational repression are unknown. Some details of these processes have been elucidated in yeast, neuroblastoma cells or cultured oligodendrocytes, but there is good reason to expect that, at least at the basic level, the same molecular mechanism pertains for primary neurons [114].

Arguably the best-studied example of translational control of RNA localisation is the trafficking of the mRNA encoding β-actin. ZBP1 localises with β-actin mRNA co-transcriptionally [115], leading to assembly of a localised actin mRNA-ZBP1 complex in the nucleus which is translationally silenced when it reaches the cytoplasm. The localisation of the repressed granules to sites of actin polymerisation modulates cell migration during embryogenesis, differentiation and axon guidance [116]. ZBP1 modulates translation, by zipcode-dependent blockage of translation initiation, promoting translocation of this RNA to actin-rich protrusions in fibroblasts and neurons. This inhibition of translation is lifted once the mRNA has been localised at the cell periphery where the ZBP1-mRNA complex is phosphorylated by the membrane–associated src protein kinase. This post-translational modification promotes in vivo restoration of translation by phosphorylating a key residue, Y396, of ZBP1 (in neuroblastoma cells), thereby decreasing ZBP1 binding to β-actin mRNA [115]. Src kinases and ZBP1 associate in close proximity to filipodia and growth cones of differentiated NG cells i.e. at the periphery of neurons.

Another well described but apparently more complex example, which parallels the zipcode- and ZBP-dependent transport of β-actin mRNA and phosphorylation-dependent translation of this protein by src kinase, is the trafficking and translation of A2RE11-containing mRNAs in oligodendrocytes and neurons. These RNAs bind hnRNP A2, a protein that is expressed at a high level in these cells and is incorporated along with A2RE-containing mRNAs into transport granules that accumulate in the soma and dendrites [8, 117]. In oligodendrocytes these granules contain myelin basic protein (MBP) mRNA, an A2RE-containing mRNA that is associated with TOG, a 218 kDa, MT-associated protein possessing clusters of multiple HEAT (Huntingtin, Elongation factor 3, the PR65/A subunit of protein phosphatase 2A and the lipid kinase Tor) repeats. In oligodendrocytes, translation of MBP mRNA within the granules may be suspended during trafficking by the binding of hnRNP E1 with dimers of hnRNP A2, which in turn are accompanied by TOG [4]).

hnRNP E1 is one of several molecules that inhibits translation of MBP mRNA. It acts by blocking recruitment of the 60S subunit of the ribosome in haematopoietic cells [118]. In neurons, it suppresses translation of mRNAs that bear an A2RE or A2RE-like *cis*-acting motif during transport [119]. At the myelin-forming periphery of oligodendrocytes and in the dendrites of neurons hnRNP E1 is phosphorylated in vivo by an unidentified kinase. This modification activates translation by an unknown mechanism and may involve dissociation of hnRNP E1 from the trafficking granule because of phosphorylation, some other post-translation modification (E. Barbarese and J. H. Carson, personal communication) [4, 119] or a phosphorylation-regulated change in subcellular distribution of E1. However, as discussed above, E1 is not the only inhibitor of MBP mRNA translation. The src kinase-induced phosphorylation by ZBP is paralleled by the phosphorylation of hnRNP A2 by fyn kinase, at its cellular destination, leading to translation of MBP mRNA, which is needed in oligodendrocytes to form myelin at the cell periphery [120]. More recently, hnRNP F has also been implicated in this pathway [121].

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FMRP also regulates translation of mRNAs that have been transported in granules. FMRP which has been phosphorylated by the ribosomal S6 kinase-1 (S6K1) kinase inhibits translation of many of the mRNAs in granules during trafficking, with dephosphorylation enhancing translation of target mRNAs such as SAP90/PSD-95-associated protein 3 by association with actively translating ribosomes [122, 123]. Not surprisingly, it has been shown that as the FMRP mRNA levels decline in oligodendrocytes MBP translation rises. FMRP binds MBP mRNA simultaneously with hnRNP E1 binding A2 [124]. It has been suggested that FMRP may fill or share the role previously ascribed to hnRNP E1: inhibiting translation of RNAs containing A2RE or A2RE-like motifs during cytoplasmic transport, but not of RNAs lacking this element. It is envisaged that each of the HEAT-repeats of TOG binds a dimer of hnRNP A2, which links an A2RE-containing transcript to TOG, and a copy of FMRP, which prevents translation during transport but subsequently generates scaffolding protein components of the postsynaptic density [125].

The microtubule-associated protein TOG2 (tumour overexpressed gene2, the larger, alternatively spliced isoform of TOG) binds hnRNP A2, and regulates the direction of granule transport [52, 126]. TOG is co-localised with hnRNP A2 and thus with A2RE-mRNA in oligodendrocytes (and B104 neuroblastoma cells): it appears to mediate association of hnRNP A2-positive granules with microtubules in trafficking or localization [127]. shRNA directed at TOG did not affect assembly, trafficking, or localisation of granules when TOG expression in a neural cell line was reduced. TOG appears to be necessary for efficient translation of MBP mRNA and this role is probably mediated by TOG interaction with hnRNP A2 [126].

Several other mechanisms for repression of translation in RNA trafficking granules have been proposed [6] but the molecular mechanisms of de-repression are still largely unexplored. Synaptic activation results in translocation of mRNA from RNA granules to polysomes e.g. for Staufen-containing granules [59]. RNA-associated CPEB binds maskin, which also binds the eukaryotic translation initiation factor (eIF4E) (Fig. 4). The mRNA cap-binding complex has three subunits: eIF4E, the cap-binding protein; eIF4A, an RNA helicase; and eIF4G, a modular scaffold protein. eIF4G binds eIF4E, eIF4A and the poly(A) binding protein (PABP). In this complex, eIF4E does not bind eIF4G, a step required for initiation of translation. Translational repression of CPE-containing mRNAs can be reversed by stimulation of NMDA receptors. Receptor activation leads to CPEB (a conserved zinc finger and RRM mosaic protein) phosphorylation by the kinase Aurora A, causing dissociation of maskin from eIF4E, the cleavage and specificity factor (CPSF) recruitment, polyadenylation and thereby, translation initiation [11]. The CPE has the consensus sequence AAUAAA and is found in the 3′ UTRs of most mRNAs. Binding to this element controls translation by cytoplasmic polyadenylation. CPEB is regulated through phosphorylation by the kinase Eg2: this phosphorylation results in recruitment of CPSF to the AAUAAA element [128]. A second sequence element, UUUUUAU, activates poly(A) addition during maturation. UUUUUAU and AAUAAA are both necessary and sufficient for maturation-specific polyadenylation [129].

Another model for lifting the suppression of translation involves the *trans*-membrane receptor, DCC (deleted in colorectal cancer), which associates with the protein synthesis machinery and regulates translation [130]. DCC is a receptor for the extracellular factor netrin that is known to promote protein synthesis in axons. By co-immunoprecipitation, electron microscopy and immunofluorescence Tcherkezian and colleagues were able to visualise a physical complex of DCC with components involved in translation initiation, including eIFs, ribosomal subunits, and monosomes. Furthermore, DCC mediated translational regulation in response to its ligand netrin-1. The authors put forward a generalizable model with *trans*-membrane association of cell surface receptors and the translation machinery contributing to the specificity, efficiency, and spatially precise control of translation, based on a transmembrane complex regulated by extracellular cues.

Stress granules to neuronal mRNP, what's the difference?

Stress granules (SG) are specialised mRNP complexes that form in the cytoplasm in response to external stimuli such as oxidative stress, heat shock, glucose deprivation, and viral infection (reviewed in [131–133]). Inappropriate assembly of SG may cause neuronal disorders such as amyotrophic lateral sclerosis [134] and other neurodegenerative disorders (reviewed in [135]). Functionally they are speculated to triage mRNA translation and stability with a view to mediating gene expression so that the cell can respond to or survive its changed environment. In vitro this can be visualised by the formation of non-membrane-bound granules forming in the cytoplasm after the induction of stress. In general, SG contain stalled 48S preinitiation complexes [136] and typical markers for SG include early initiation factors eIF2, eIF3 and eIF4E. However, researchers use T cell intracellular antigen 1 (TIA-1) or G3BP1 (G3BP are reviewed in [137]) to visualise SG by fluorescent microscopy. Other components of SG can include PABP, TIA-1 (T-cell intracellular antigen-1), TIA receptor (TIAR), HuR, Staufen, and FMRP along with others [132]. Many of these components are shared with neuronal mRNP which presents a problem of how to determine

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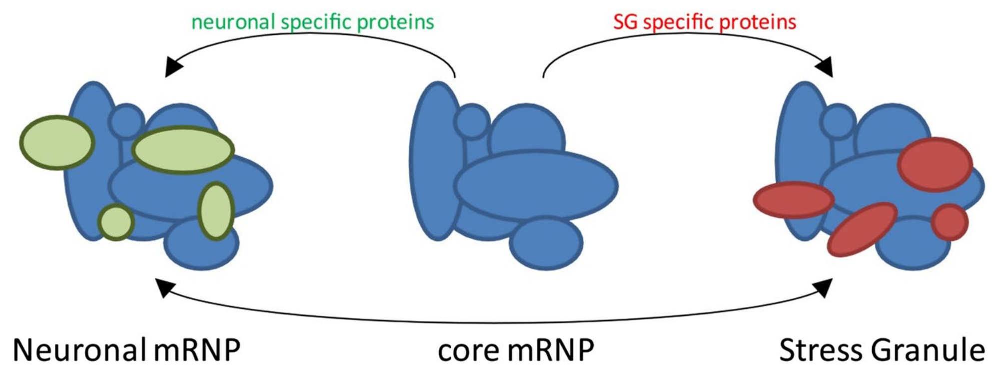

Fig. 5 The continuum of mRNPs. The difference between neuronal mRNP and stress granules is dynamic with shared components making the core mRNP, proteins are added and subtracted from the complexes to respond to cellular demands and function. In this model it may not be possible to define the differences between an mRNP and Stress granules or other RNA-protein complexes

the difference between SG and neuronal mRNP. This was made more evident with the report by Tazi and co-workers [138] that deficiencies of G3BP1 leads to abnormal synaptic plasticity. The definition of what makes up these particles in not clear, because they appear to be part of a continuum rather than multiprotein complexes with a discrete function. Evidence suggests that some common proteins are used to seed the formation of these particles, including commonly used markers such as TIAR, FMRP and G3BP1 although some redundancy may exist between G3BP1 and G3BP2 [139]. However, recent findings suggest that even some of the components that are considered essential are indeed redundant, for example, Jedruskik-Bode and colleagues used G3BPKO MEFs and were still able to induce SG formation. Other proteins appear to be transient through the progression from neuronal mRNP to SG and may include regulatory proteins such as receptor for activated C kinase 1 (RACK1) which signal proteins to leave or join the particle or to initiate processing of the mRNA contained within the particle (reviewed in [140]). It may be incorrect to consider mRNP as independent entities, such as neuronal mRNP and SG, considering their true dynamic nature (Fig. 5). Indeed Dewey and colleagues propose that SG are cell type specific responding to different stresses [141]. They suggest that sodium Arsenite will cause trans-active response DNA binding protein 43 kDa (TDP-43) containing SG formation in HeLa cells but not SH-SYSY cells, conversely Arginine may stimulate formation of TDP-43 containing SG in SH-SYSY cells but not HeLa cells. Undoubtedly, this will cause nomenclature problems as different groups continue to report their functions in different tissues in response to varying environmental stimuli. For example, nuclear protein SIR-2.4 (mammalian homolog of SIRT6), was originally thought to only influence nuclear biology. However, it has recently been reported to regulate SG formation in the cytoplasm and the authors speculate that this may be a mechanism to regulate both nuclear and cytoplasm responses to stress [142]. It would appear that many proteins signal by direct interaction with components of SG and SMN (reviewed below) is one of them [143]. The range of proteins reported to reside or transit into SG makes it difficult to define the exact nature of SG and further blurs the distinction between SG and neuronal mRNP.

Case studies of RNA-binding proteins in human disease

FMRP control of translation of mRNAs in granules

Given the central role played by FMRP and its binding partners (Table 1) in trafficking granules in the brain it is not surprising that the absence of this protein results in profound phenotypic change. FMRP is encoded by the *FMR1* gene, which normally contains 5–45 repeats of the base sequence CGG [144] and expansion of this triplet repeat is correlated to disease onset. FMRP is not only associated with RNA trafficking granules, translation in dendritic spines, and memory (discussed earlier), but also with two well-recognised syndromes that are a major cause of intellectual disability. Fragile X syndrome (FRAXA) results from a lack of expression of FMRP (reviewed in [145, 146], brought about by a marked CGG repeat expansion (to greater than 200 copies) in the *FMR1* gene, resulting in changes in dendritic spine morphology [147]. A related syndrome, fragile X-associated tremor/ataxia syndrome (FXTAS), arises from over-expression of premutant *FMR1* mRNA: the premutant also has a repeat expansion, but less marked (55–200 repeats) than the full mutation, that causes FRAXA [106].

FMRP has several modes by which it can regulate translation. Studies of FRAXA highlight the effects of localised translation on neuronal structure and function. FMRP-containing trafficking granules migrate into dendrites when Group-1 metabotropic glutamate receptors (mGluRs) are activated [17]. mGluRs are strong activators of translation and their stimulation induces rapid synthesis of FMRP in the synapto-dendritic compartments [148–151].

Almost all of the FMRP in NIH3T3 and HeLa cells is associated with mRNA in actively translating polyribosomes [152]. Using an *FMR-1* knockout (KO) mouse,

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Table 1 FMRP interacting proteins in neuronal function

Protein partner	Cellular localization	Proposed function	References
FXR1P	Nucleus and cytoplasm	Transport/translational control of dendritic mRNA	[181]
FXR2P	Nucleus and cytoplasm	Transport/translational control of dendritic mRNA	[182]
NUFIP1	Cytoplasm	Export and localization of mRNA	[183]
CYFIP1 and CYFIP2	Cytoplasm	Control active cytoskeleton dynamics	[184]
82-FIP	Nucleus and cytoplasm	RNA metabolism (maturation, storage, mRNP assembly)	[185]
Nucleolin	Nucleolus and cytoplasm	mRNP formation or nucleocytoplasmic shuttling or translational control	[186]
YB1/p50	Cytoplasm	mRNA translation and regulation	[187]
Staufen	Cytoplasm	Regulation of mRNA transport and translation	[188]
Pur −α	Cytoplasm	Regulation of mRNA transport and translation	[189]
Myosin Va	Cytoplasm	Translocation of mRNP	[190]
IMP-1	Cytoplasm	mRNA transport	[191]
Ran BPM	Cytoplasm	Microtubule organisation	[192]
Lg1	Cytoplasm	mRNA sorting, transport and anchoring	[193]
KIF5a	Cytoplasm	Transport of RNA granules in dendrites	[58]
MSP58	Nucleus and cytoplasm	RNP synthesis and translation regulation	[194]

The table shows known protein binding partners of FMRP along with the subcellular localisation of the interaction and the potential role the interaction has in regulating RNA transport or translation

Aschrafi and co-workers [153] demonstrated, in adult mouse brain, that actively translating polysomes might be derived from translationally silent FMRP-containing granules. Loss of FMRP in FRAXA results in activation of translation and the accompanying characteristic deformation of neuronal spines [154–156]. By contrast, FMRP over-expression results in an increase in stalled polysomes if this protein is phosphorylated, i.e. dephosphorylation may release the polysomes from the stalled state [157]. Recently, Narayanan and co-workers [123] identified S6K1 and protein phosphatase 2A (PP2A) as key regulators of FMRP phosphorylation which appear to turn translation of FMRP-bound mRNA on or off. FXR2, which is closely related to FMRP, is also found in active polyribosomes [152]. Although it is well understood that mGluRs mediate local mRNA translation in synaptodendritic domains, the details of the molecular mechanism of stimulation are unknown, though recent findings from the Greenough laboratory [158] have shed some light in this direction. 0–40 min after mGluR5 stimulation, granules containing GFP-labelled *Fmr1* and *CaMKIIα* mRNAs undergo slower motion in wild-type neurons but not in *fmr1* KO mice. 40–60 min after stimulation, the mRNAs regain their former motion. Moreover, *CaMKIIα* mRNA can be delivered and translated in dendritic spines of wild-type neurons within 10 min in response to group I mGluR stimulation, whereas KO neurons fail to show this response. 20 min following stimulation, FMRP was translated close to mGluR5 whereas *CaMKIIα* mRNA and protein synthesis were increased at dendritic spines in wild type but not neurons of KO mice. These data suggest that FMRP may be involved in mediating spatial mRNA delivery for local protein synthesis in response to synaptic stimulation. In addition, Pfeiffer and Huber [159] demonstrated that wild-type expression of FMRP decreases the number of functional synapses in cultured neurons when compared to *Fmr1* KO cells, implying that the correct location and expression of FMRP-bound RNAs may be responsible for normal neuronal development.

The downstream signalling involved in mediating the connection between synaptic stimulation and FMRP is partly understood [160]. RACK1 is a component of mRNP complexes and together with PKCβ2, RACK1 associates with β-tubulin in mRNP and its binding to mRNP is controlled by the stimulation of mGluR activation in hippocampal neurons, providing some insights into the signalling pathway involved in regulating translation from these granules. These findings also exemplify the receptor-induced changes in activation, accessibility and composition of the mRNP components. These data suggest that FMRP is a translational regulatory protein for mGluR-mediated signalling in long-term depression (LTD) and elucidates some of its potential physiological roles.

A focus on SMN as an mRNP component

Survival of motor neuron protein (SMN) is discussed here as a component of an mRNP that is assembled in the nucleus. Assembly of mRNPs starts in or around the nuclear speckles, the RNA processing domains in the nucleus where splicing of pre-mRNA takes place (reviewed by [161]). Several components of the mRNPs carry out

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biological functions in the cytoplasm. Many proteins demonstrating dual functions in spliceosomes and mRNPs have been identified and one protein of particular interest, because of its association with disease, is SMN. The SMN gene is localised as an inverted repeat on chromosome 5q13 in humans (reviewed in [162]). Two SMN genes (SMN1 and SMN2) are expressed. In patients with a deletion of the chromosomal region encoding SMN1 all proteins are expressed from the SMN2 gene. The SMN2 gene contains a mutation in exon 7 which influences splicing of that same exon giving rise to the majority of transcripts encoding the relatively unstable SMNΔ7 protein. SMN2 does generate some full-length SMN but it is not enough to compensate for the loss of SMN1 (reviewed in [162]). The result of these defects gives rise to the neurodegenerative disease spinal muscular atrophy (SMA). SMN1 is expressed in all tissues [163] with high levels within the central nervous system, especially the spinal cord [164]. SMN1 is known to be associated with a diverse set of ribonucleoproteins (RNPs) that are targeted to subnuclear structures, such as Cajal bodies [165], and participate in spliceosome assembly and pre-mRNA splicing [163]. Besides its association with spliceosomes, SMN1 associates with cytoskeletal filaments [166] and polyribosomes [167] in the cytoplasm. SMN, along with Gemin2, is localised in dendrites as well as in axons [166]. Gemin2 is one of a group of seven related proteins, the Sm-proteins, that are involved in maturation of small nuclear RNA (snRNA). Fluorescence microscopy, digital imaging, and quantitative analyses of live and fixed neurons has shown that an SMN-Gemin2 complex is present in neuritic granules (Fig. 6) [168]. The structure of SMN bound to Gemin2 has been elucidated and reveals insights into how SMN is engage in binding to small nuclear ribonuclear particles (snRNP) and their biogenesis [169]. In vivo, in spinal cord motor neurons, SMN is actively transported in neurites and growth cones in a microtubule-dependent, bi-directional manner [168]. SMN is actively transported to the neurite outgrowths and researchers have hypothesised that the exon 7 deletion in this protein causes impairment in normal intracellular trafficking of SMN leading to SMA [170]. Over-expression SMNΔ7 showed abnormal accumulation of SMN in the nucleus and reduced neurite growth [168]. Interestingly, despite a ubiquitous function of SMN in spliceosome assembly, the exon-7 defect is implicated only in neurons, suggesting a distinct function for SMN in neurons [168].

In mice, there is a single copy of the SMN gene and the deletion of this causes embryonic lethality [171]. Therefore the mouse model is popular for modelling the most sever type of disease. This is done by deleting of the mouse Smn gene and addition of a human SMN2 transgene (*Smn−/−;SMN2*).

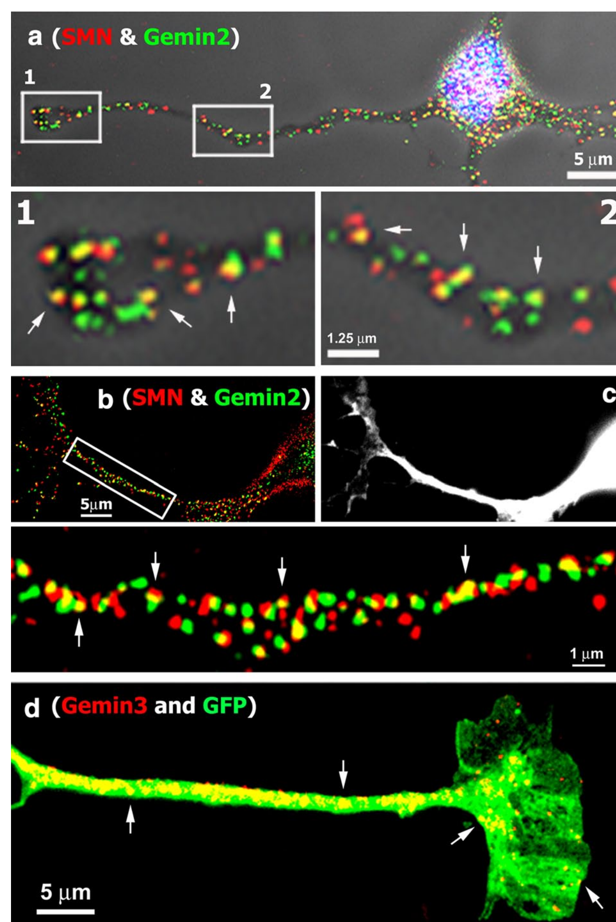

Fig. 6 Colocalization of endogenous SMN and Gemin proteins in neurites and growth cones in primary forebrain culture and ES cell-derived motor neurons. **a** SMN (*red*) and Gemin2 (*green*) in cultured forebrain neurons (3 DIV) were detected by double-labelled immunofluorescence using a monoclonal antibody to SMN and polyclonal antibody to Gemin2. The nucleus was stained with DAPI (*blue*). Higher magnification of two regions (*insets 1*, *2* from *top panel* are enlarged in *bottom panel*) depicts the frequent colocalization between SMN and Gemin2 within granules in the growth cone (*1*, *arrows*) and neurite (*2*, *arrows*). **b** Double-labelled IF showing colocalization of SMN (*red*) and Gemin2 (Cy5 antibody displayed in *green*) in neurites of ES cell-derived differentiated motoneuron. Higher magnification of a boxed region depicts numerous granules with colocalization between SMN and Gemin2 (*bottom panel*, *arrows*). **c** These cells express EGFP from a motor neuron-specific promoter. **d** IF detection of Gemin3 with a monoclonal antibody depicts many granules localised to the EGFP-positive axon and growth cone of the motor neuron (*arrows*). Reproduced with permission of the *Journal of Neuroscience* from [168]

It has been suggested that there is a motor neuron specific additional role of the SMN protein. Studies on cultured embryonic mouse motor neurons have shown that SMN is not only present in the nucleus but also in the cytoplasm and growth cones [74]. The Smn deficient motor neurons have shorter axons when cultured on extra cellular matrix laminin111 as compared to the wild type motor

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neurons [74]. In a yeast two hybrid screen for interaction partners of SMN, the RNA binding protein hnRNP-R was identified which and found to be associated with SMN in the growth cones of cultured motor neurons [172]. The over expression of hnRNP-R was found to rescue the in vitro phenotype of reduced axon length of the Smn deficient motor neurons [74]. It was also observed that the SMN hnRNP-R complex associates with β-actin mRNA in the growth cones of motor neurons [173]. The in vivo studies on the hnRNP-R knock down in zebra fish using morpholinos showed shorter axons and increased branching of the motor axons [75] which mimics the phenotype of Smn knock down by morpholinos in zebra fish [174]. Further studies to analyse the local translation of β-actin mRNA in the *Smn−/−;SMN2* motor neuron growth cones revealed that there is decreased local translation of β-actin mRNA in *Smn−/−;SMN2* motor neurons when cultured on laminin111 whereas when the *Smn−/−;SMN2* motor neurons are cultured on laminin211/221, there is increased translation of β-actin mRNA [175]. This is an interesting observation because it shows that it is not the reduced availability of the β-actin mRNA, but regulatory mechanisms for local translation that are defective in Smn-deficient motor neurons. These results are important for the understanding of the patho-physiology of this disease. Knock down of the protein PTEN (phosphatase and tensin homolog), a negative regulator of the mammalian target of rapamycin (mTOR) pathway, is able to rescue the axon growth defects and improves the survival of the motor neurons [176]. These results indicate that SMN is not only involved in nuclear RNA processing but is also engaged in the regulation of the local translation of β-actin mRNA which is critical for the disease pathology. In relation to SMN's other binding partners, hnRNP Q has been implicated in regulation of mRNA stability [177], editing [178] and splicing [179]. Thus SMN directly or along with its binding partners plays a crucial role in the regulation of all the steps of mRNA processing starting from splicing, to mRNA stability, transport and regulation of translation.

Conclusions

Neuronal Translation, do we get the message? We do, but the message is considerably more complex than our original picture. mRNA is carefully regulated from transcription through to spatial and time-specific translation under the control of a myriad of internal and external cues. It is not surprising that mental diseases are so intricate, with familial diseases often displaying defects in several genes within a known signalling pathway. Add to that the complexity of the mRNA metabolism described here and we gain some feeling for the difficulties that confront research in the field. It will take many years before we fully appreciate how these processes influence the aetiology of mental disorders. The paths followed by mRNA in oligodendrocytes and neurons show considerable overlap and raise the possibility that other CNS cell types, especially astrocytes, take the same steps from the nucleus to the cell periphery. These pathways involve proteins of particular interest, including the hnRNP proteins, which possess multiple functions. Several of these proteins have normal and pathological roles, spanning from the involvement of CaMKIIα in normal memory formation to the aberrant behaviour of FMRP in Fragile X and other mental disorders. There is considerable scope for exploration of the functions of these proteins to fully understand how we should process the message (RNA).

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