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Neuronal RNA Granules: Movers and Makers

Minireview

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RNA localization contributes to cell polarity and synaptic plasticity. Evidence will be discussed that RNA transport and local translation in neurons may be more intimately linked than originally thought. Second, neuronal RNA granules, originally defined as intermediates involved in mRNA transport, are much more diverse in their composition and functions than previously anticipated. We focus on three classes of RNA granules that include transport RNPs, stress granules, and P bodies and discuss their potential functions in RNA localization, microRNA-mediated translational regulation, and mRNA degradation.

Translational control of localized mRNAs is a common mechanism for regulating protein expression in specific subdomains of a cell. It plays an important role in a number of processes, such as the formation of the body axes, asymmetric cell division, and cell motility (St Johnston, 2005). In neurons, localization of mRNAs at the synapse has been proposed as a mechanism for synaptic plasticity and thus learning and memory (Klann and Dever, 2004). Recently, it has also been shown that mRNA targeting and local protein synthesis can influence axon guidance and nerve regeneration (Willis et al., 2005). It is now generally accepted that localized mRNAs are often transported in large ribonucleoprotein particles (RNPs), which have been referred to as RNA granules (Ainger et al., 1993; Knowles et al., 1996; Köhrmann et al., 1999; Kiebler and DesGroseillers, 2000). Interestingly, there may be alternative models for RNA localization. In the *Drosophila* egg, *hsp83* mRNA can be localized by degrading all transcripts that are not correctly localized, and transcripts can also become localized by passively diffusing through the cytoplasm until they are locally anchored (St. Johnston, 2005, and references therein). In yeast, ER tubules are involved in the localization of *ASH1* mRNA to the bud, suggesting a myosin-dependent cotransport of tubules and localized RNPs (Schmid et al., 2006). In this review, we focus on progress to define RNPs involved in mRNA localization, sorting, and degradation.

Significant progress has been made in identifying components of such localized RNPs. A thorough comparison revealed that some RNP components are conserved across species and used in different cellular contexts (Kiebler and DesGroseillers, 2000, and references therein). However, very few of these proteins are essential for RNA localization in polarized neurons. This leaves us with several important questions. First, are there different types of RNA granules for different RNAs, or do all RNPs contain an obligatory subset of *trans*-acting factors? Second, how are RNAs translationally repressed until they reach their final destinations? Do these neuronal RNA granules, for example, contain microRNAs that silence the transported messages? Third, how do transport RNPs relate to stress granules and processing bodies, two recently discovered RNA granules in mammalian cells? Are these different RNA granules invariantly particles with specific functions and independent assembly pathways, or are these dynamic structures that share components or even interconvert? We will focus on neuronal RNA granules and review the evidence for their role in neuronal RNA transport, translational regulation, and possibly in RNA processing.

The Discovery of Transport RNPs

Many in situ hybridization studies (ISH) studies had previously revealed the nonrandom localization of specific mRNAs to subcellular domains of polarized cells, such as yeast, oocytes, fibroblasts, and neurons (St. Johnston, 2005). However, these studies only provided information on the steady-state distribution of mRNAs, and alternative approaches were needed in live cells to understand the dynamic process of mRNA localization. In a seminal study, Ainger et al. (1993) fluorescently labeled and microinjected *myelin basic protein* (*MBP*) mRNA into cultured oligodendrocytes. The *MBP* mRNA formed granules, which were rapidly transported along microtubules (MT) into processes at a rate of 0.2 μm/s. The observed RNA granules were heterogeneous in size and displayed persistent, oscillatory, or immobile characteristics. This work represented the first characterization of mRNA movements in living cells and suggested RNA granules as intermediates in a multistep pathway to localize mRNAs (Ainger et al., 1993). Wilhelm and Vale were the first to call them "RNA transport particles" (Wilhelm and Vale, 1993); we therefore refer to them as transport RNPs.

In neurons, the first evidence for the existence of transport RNPs (Table 1) came from Knowles et al. (1996). The fluorescent vital RNA dye SYTO14 depicted the dynamic movements of endogenous RNA granules into dendrites of cultured cortical neurons. These transport RNPs displayed rapid anterograde and retrograde trajectories that were dependent on MTs, consistent with an active transport process (Knowles et al., 1996). Furthermore, they contained many translational components, such as elongation factors and ribosomal proteins or even clusters of ribosomes, supporting the previous findings on *MBP* mRNA granules. These findings led to the hypothesis that transport RNPs move as discrete units together with at least some of the machinery needed to initiate translation upon reaching their final destination.

This leads us to an important question: are RNA granules homogeneous in composition and function, or do various types exist? Evidence for the latter hypothesis

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Table 1. Diversity of Cytoplasmic RNA Granules in Neurons

Name	Synonyms	Definitions	Key Components	Key References
Transport RNPs	Neuronal RNA granules, RNA particles	Motile granules transporting mRNA. Contain translational components. mRNAs in these RNA granules are translationally arrested during transport by the action of regulatory RNAs and RNA-binding proteins. Biochemical evidence for RNA granules with and without ribosomes.	Staufen1, Staufen2, FMRP, ZBP1, hnRNPA2, CPEB, Purα, SMN	Knowles et al., 1996; Köhrmann et al., 1999; Zhang et al., 2001, 2006; Tang et al., 2001; Huang et al., 2003; Kanai et al., 2004
Stress granules	Heat stress granules	Harbor translationally arrested mRNAs that form in cells exposed to a broad range of stresses. Sort, remodel, and export specific RNAs for reinitiation or storage.	TIA-1, TIAR, PABP, G3BP, 40S ribosomal subunit	Anderson and Kedersha, 2006; Vessey et al., 2006
Processing bodies (P bodies)	Cytoplasmic bodies, Dcp1 bodies, GW bodies	Sites of translational repression and/or mRNA degradation. Contain RISC machinery including microRNAs. Do not contain ribosomal subunits.	Dcp1a, Lsm proteins, Rck/p54, GW182	Anderson and Kedersha, 2006; Schratt et al., 2006; Vessey et al., 2006

Since the first description of motile transport RNPs in oligodendrocytes and neurons, at least two additional classes of neuronal RNA granules exist: stress granules and P bodies that are sites for RNA storage and degradation, respectively. With regard to possible shared components between these neuronal RNA granules, there is evidence that several mRNA binding proteins, i.e. staufen1, staufen2, FMRP, SMN, CPEB, and pumilio2, can associate with SGs upon overexpression (Anderson and Kedersha, 2006). The presence of components in more than one type of RNA granule suggests that these RNPs are not homogeneous, but represent dynamic structures that a cell uses to sort mRNAs and regulate their translation and degradation.

came from several laboratories that have identified mRNA binding proteins involved in mRNA localization in polarized cells. One key player is Staufen, a double-stranded RNA binding protein that plays important roles in localization of *bicoid* and *oskar* RNA to the anterior and posterior pole, respectively, in *Drosophila* oocytes (St Johnston, 2005). Köhrmann and colleagues were the first to express the mammalian Staufen homolog tagged with GFP in hippocampal neurons and to observe the MT-dependent recruitment of Staufen into RNA granules, which exhibited rapid bidirectional movements in dendrites (0.1–0.4 μm/s). This was the first demonstration of dynamic movement of an RNA binding protein in transport RNPs in living neurons (Köhrmann et al., 1999). This approach was subsequently used by Zhang and colleagues to analyze the movement of granules (over 1 μm/s) containing the *β-actin* mRNA binding protein zipcode binding protein 1 (ZBP1) into growth cones of developing axons (Zhang et al., 2001). Furthermore, anterograde trafficking of RNA granules containing ZBP1 and *β-actin* mRNA was stimulated by the neurotrophin NT-3, suggesting that granules can be regulated by physiological signals. Rook and colleagues took a different approach to visualize RNPs in neurons by employing the MS2-GFP tagging system to show that movement of granules containing the 3′-UTR for *CaMKIIα* mRNA in dendrites is dependent on synaptic activity (Rook et al., 2000). Neuronal depolarization increased the fraction of the *CaMKIIα* reporter mRNA that was in the anterograde motile pool, suggesting that oscillatory granules were capable of sampling multiple nearby synapses. These finding indicate a possible role of synaptic input in the regulation of RNA granule motility in dendrites and localization at synapses. Taken together, these studies underscore that not only are mRNAs packaged into transport RNPs for directed movement but that there are signaling mechanisms likely regulating distinct populations of these transport RNPs and their dynamic interrelationships.

Live cell imaging studies are consistent with the notion that transport RNPs may be propelled by molecular motors. First, MT-depolymerizing drugs were shown to decrease the levels of RNAs or mRNA binding proteins in neuronal processes (Knowles et al., 1996; Köhrmann et al., 1999; Rook et al., 2000). Second, the observed velocities of RNA granules (0.2–1.5 μm/s) and their anterograde or retrograde trajectories over long distances further suggest that they depend on kinesin and dynein motors. Indeed, previous work from the Carson group showed that antisense knockdown of the conventional kinesin heavy chain (KIF5b) impaired the ability of microinjected *MBP* mRNA to translocate into oligodendrocyte processes. Kanai and colleagues found that KIF5b associates with large RNA granules that contain 42 proteins and two well-studied dendritically localized mRNAs, *CaMKIIα* and *Arc* (Kanai et al., 2004). Overexpression of KIF5b increased mRNA localization into distal dendrites, whereas mRNA localization was reduced following its knockdown. Further work is needed to define the specific molecular interactions between transport RNP components and kinesin subunits to enable directed RNA movement. Nonetheless, this study provides compelling evidence that the anterograde transport of at least some transport RNPs is mediated by conventional kinesin in hippocampal neurons. It will also be important to study how transport RNPs that are capable of bidirectionally trafficking may become selectively captured within stimulated dendritic spines.

Based on the landmark findings of Carson and others, a compelling model has been put forth of how mRNA

localization could be achieved in an ordered, multistep pathway (Wilhelm and Vale, 1993). It predicts (1) the formation of transport RNPs as a functional complex, (2) their motor-dependent translocation to their destinations, (3) their anchoring to the local cytoskeleton, and (4) the translational derepression of the localized mRNAs. Since the formulation of this model, the challenge for the last 15 years has been to identify the key players involved in these processes and to understand these two processes at the molecular level.

Key Components of Neuronal Transport RNPs

First attempts have been made to purify neuronal RNA granules in order to utilize proteomics to characterize the molecular composition of neuronal RNA granules in a more systematic manner and to begin validating their functional significance in RNA transport. First, Krichevsky and Kosik (2001) identified an unusually heavy sucrose gradient fraction beyond polysomes that contained ribosomes and Staufen1 and are believed to represent a fraction enriched for components of RNA granules. Kanai et al. (2004) isolated large *CaMKII*α and *Arc* RNA-containing granules from adult mouse brain that associate with KIF5. Using the RNA interference (RNAi) approach, they showed that four of the proteins identified were important for the localization of a *CaMKII*α reporter: Purα, hnRNP U, polypyrimidine tract binding protein-associated splicing factor (PSF), and Staufen1. SYNCRIP (hnRNP-Q1) was another mRNA binding protein identified, although its knockdown in neurons had no effect on *CaMKII*α mRNA localization. Also identified in this study was the fragile X mental retardation protein (FMRP), an mRNA binding protein that traffics in RNA granules in dendrites (Antar et al., 2004). Taken together, the study by Kanai et al. was groundbreaking in three ways. First, it showed that conventional kinesin is involved in the transport of RNA granules to dendrites, although it is still unclear which RNP component binds directly to which kinesin subunit(s). Second, it identified with Purα, hnRNP U, and PSF three additional essential *trans*-acting factors for dendritic RNA transport. Third, it strongly suggested that granules are composed of many mRNA binding proteins and a number of these may not be essential for mRNA transport, but instead regulate aspects of RNP assembly, translation, or stability.

Elvira et al. (2006) biochemically isolated RNA granules in developing rat brain that were enriched for β-*actin* but not for *CaMKII*α mRNA and contained ribosomes, a large set of RNA binding proteins, MT-associated proteins, and several novel proteins. One protein identified was ZBP1, which was known to be required for localization of β-*actin* mRNA granules (Zhang et al., 2001). In addition, Staufen and hnRNP-A2 were identified as also being required factors for mRNA localization. Interestingly, both proteomic studies identified a number of RNA binding proteins, such as Staufen and SYNCRIP, which was shown by Bannai and colleagues to colocalize with GFP-tagged Staufen1 and *inositol 1,4,5-trisphosphate receptor type 1* mRNA in dendritic granules (Elvira et al., 2006, and reference therein). Another noteworthy protein family detected in both studies is the family of DEAD box helicases, which has previously been implicated in RNP assembly. Elvira et al. (2006) observed that DEAD Box 3 colocalized with RNA granule markers and also exhibited BDNF-regulated movements in live hippocampal neurons.

There were also some interesting distinctions between these two studies. Elvira et al. (2006) identified ZBP1 in their proteomic analysis of developing brain, whereas Kanai et al. (2004) did not. The authors hypothesized that there may be different types of RNA granules that are developmentally regulated and respond to distinct physiological signals. Together, these studies open the door toward a more complete understanding of the composition of neuronal RNA granules and allow us to address some exciting new questions. One shortcoming of these studies, however, is that very few components that came out of these proteomics experiments have been validated in RNA localization assays, and it is unclear up to now whether the majority of the identified components are indeed part of transport RNPs.

What is the experimental evidence for an involvement of *trans*-acting factors in RNA localization? The most-studied example is ZBP1 and its role in the localization of β-*actin* mRNA into neurites and growth cones (Zhang et al., 2001). Antisense oligonucleotides directed against the 54 nt β-*actin* zipcode, which disrupt ZBP1 binding to the zipcode in vitro, blocked the NT-3-induced localization of β-*actin* mRNA into neurites and growth cones (Zhang et al., 2001). Furthermore, morpholino antisense oligonucleotides to knock-down ZBP1 resulted in reduced dendritic β-*actin* mRNA localization in cultured hippocampal neurons, whereas the localization of *CaMKII*α mRNA was unaffected (Eom et al., 2003). Another *trans*-acting factor that has been implicated in dendritic RNA transport has been mammalian Staufen2. Overexpression of dominant-negative Staufen2 significantly reduced the level of ethidium bromide-stained RNA in dendrites of polarized neurons (Tang et al., 2001). Unfortunately, there is still not a single mRNA identified in mammalian cells that is recognized by Staufen proteins and that has been shown to be transported within Staufen-containing RNA granules. This is in sharp contrast to *Drosophila* Staufen, which is involved in the localization of *bicoid*, *oskar*, and *prospero* RNAs in the *Drosophila* oocyte and embryo (St Johnston, 2005). Finally, a recent study reported an essential role of the cytoplasmic polyadenylation element binding protein 1 (CPEB1) in dendritic RNA transport. Huang et al. (2003) showed that functional but not mutated CPEs within the 170 nt 3′-UTR of wild-type *CaMKII*α mRNA are sufficient to target a reporter RNA into dendrites of hippocampal neurons. To demonstrate a requirement for CPEB1 in dendritic mRNA localization, cultured neurons from CPEB1 knockout mice showed reduced localization of EGFP reporters harboring CPE sequences in the 3′-UTR. The study by Huang et al. (2003) showed that endogenous mRNAs that contain a CPE, e.g., *CaMKII*α and *MAP2* mRNAs, were reduced in synaptosome preparations isolated from cultured neurons that were infected with a dominant-negative CPEB construct. Reduced mRNA levels of *MAP2* mRNA were also confirmed by FISH, further suggesting a role for CPEB in mRNA localization. As other studies have identified a role for different localization elements within the *CaMKII*α mRNA 3′-UTR other than the CPE (Rook et al., 2000), it is likely that there may be additional localization elements, and further work is needed to assess whether the CPE is necessary for mRNA localization.

An aspect of mRNA localization that is as yet unclear is how transport RNPs are assembled. An RNP complex containing the survival of motor neuron protein (SMN) and gemin proteins facilitates the assembly of spliceosomal RNPs, which may play a comparable role in assembly of transport RNPs in neurons (Monani, 2005). Rossoll and coworkers have shown that SMN binds SYNCRIP (Monani, 2005, and reference therein), which was identified in the above proteomics screens of RNA granules (Kanai et al., 2004; Elvira et al., 2006). SMN-GFP granules are actively transported in association with gemin proteins in live neurons (Zhang et al., 2006, and references therein). The inherited loss of SMN is the cause of the neurodegenerative disease spinal muscular atrophy (SMA). Mouse neurons cultured from a transgenic mouse model of SMA have impaired localization of *β-actin* mRNA in axonal growth cones, suggesting possible interactions of SMN complexes with mRNA binding proteins, e.g., ZBP1 and SYNCRIP involved in mRNA localization (Monani, 2005, and references therein). Future work is needed to understand whether impaired mRNP assembly and localization contribute to SMA.

Coupling mRNA Transport with Translation for Neuronal Function

Most approaches described so far tried to understand the molecular mechanisms of how individual mRNAs are transported in the form of granules using cultured neurons as a model. The identification of various components of the translational machinery in dendrites and near synapses (Klann and Dever, 2004, and references therein) revealed several important questions. Is RNA transport coupled with translation, and how is this achieved? Are mRNAs generally repressed within the observed transport RNPs? What causes the activation of translation? In addition, more than one class of transport RNP might exist. In the case of mammalian staufen proteins, there is biochemical evidence that some RNA granules contain ribosomes, whereas others do not. When fractionated by size, the largest Staufen pools contained ribosomal and ER markers, whereas the smaller RNA granules were free of ribosomes and ER but cofractionated with conventional kinesin (Mallardo et al., 2003). This suggested that the smaller RNA granules might represent the observed transport RNPs (Köhrmann et al., 1999). Krichevsky and Kosik provided first evidence that mRNAs might be indeed translationally repressed within granules, since they can be released and/or derepressed in response to neuronal activity, allowing for local translation (Krichevsky and Kosik, 2001; see also Hüttelmaier et al., 2005).

Since this first observation, efforts were made to identify mRNA binding proteins in transport RNPs that might regulate mRNA translation. RNG105 was shown by Shiina and colleagues to be a component of dendritic RNA granules that can repress mRNA translation in vitro and in vivo (Elvira et al., 2006, and reference therein). In response to BDNF, the release of RNG105 from RNA granules was correlated with activation of mRNA reporter translation. Huang an coworkers showed that translational repression of mRNAs containing CPEs by CPEB could be derepressed by NMDA receptor stimulation, allowing for synaptic protein synthesis (Huang et al., 2003, and reference therein). This CPEB-dependent mechanism involves regulation of a multiprotein RNP complex that represses translation initiation. In response to NMDA receptor activation, CPEB is phosphorylated by aurora kinase, leading to the dissociation of maskin from eIF4E. CPSF is recruited, polyadenylation ensues, and translation is activated.

In the case of ZBP1, phosphorylation by Src led to the release of ZBP1 from *β-actin* mRNA, allowing for spatial regulation of local β-actin synthesis beneath the membrane (Hüttelmaier et al., 2005). Evidence also indicates that ZBP1 can repress the joining of ribosomal subunits, indicating regulation at the level of translation initiation. Taken together, these studies suggest that mRNA transport and translation may be inextricably linked processes through regulation of mRNA binding proteins, such as CPEB and ZBP1, constituents of transport RNPs. Future work is clearly needed to identify whether any of these mRNA binding proteins can function as an adaptor between the mRNA *cis*-acting element and the motor subunit, thus serving a dual role in the coupling of mRNA transport to translational control.

Fragile X syndrome is the most common inherited form of mental retardation caused by the loss of FMRP, an mRNA binding protein that is known to regulate translation and traffic in dendrites (Zalfa et al., 2006). FMRP granules containing mRNAs traffic into dendrites upon activation of metabotropic glutamate receptors (Antar et al., 2004), although it is still unclear whether FMRP is an essential factor for mRNA localization. Several studies indicate that FMRP plays a critical role in regulation of mRNA translation, perhaps serving as a link between transport RNPs and polyribosomes. Biochemical analysis indicates that FMRP is broadly distributed on sucrose gradients, and it is detected in both RNPs and polyribosomes (Zalfa et al., 2006). FMRP can repress mRNA translation in vitro and in vivo (Zalfa et al., 2006). One model is that FMRP, when present in polyribosomes, may repress ribosome elongation. Consistent with a stalled ribosome model, FMRP associated with polyribosomes is heavily phosphorylated, whereas dephosphorylation allows ribosomes to translate mRNA and run-off (Zalfa et al., 2006, and reference therein). FMRP within mRNPs may regulate translation by another mechanism. Some evidence indicates that FMRP can bind *BC1*, a small noncoding RNA, which may act as a bridge to deliver FMRP to complementary mRNAs (Zalfa et al., 2006). The Tiedge laboratory has characterized distinct *cis*-acting elements within the *BC1* RNA that are needed for dendritic mRNA transport and repression of translation initiation by binding PABP and eIF4A, thus inhibiting translation initiation (Wang et al., 2005). FMRP and *BC1* RNA binding was not detected by this group. A third mode of FMRP translation regulation potentially involves interactions of FMRP with microRNAs (miRNAs) and the RNA-induced silencing complex (RISC) (Zalfa et al., 2006). Further work should reveal which miRNAs associate with FMRP to regulate mRNA translation. In summary, FMRP has multiple modes of interactions to regulate translation in RNPs and polyribosomes. It will be interesting to examine specific defects in activity-dependent trafficking of transport RNPs and their translation in neurons from a mouse model of fragile X syndrome, which has emerged as a powerful model for analysis of the function of localized RNPs in specific forms of protein synthesis-dependent synaptic plasticity (Zalfa et al., 2006).

As mediators of mRNA transport and translation, some mRNA binding proteins affect neuronal morphology and function, and their deficiency may underlie genetic neurological diseases. A dendritic spine phenotype in human fragile X syndrome and the corresponding mouse model is characterized by an excess of long and thin filopodial-like spines and a reduction in mature spines, which may be due to dysregulated protein synthesis at synapses (Zalfa et al., 2006). Another prominent example is the mRNA binding protein TLS (translocated in liposarcoma) that has been previously identified as a component of neuronal RNA granules and shown to translocate into spines of hippocampal neurons in response to mGluR5 activation. Neurons cultured from TLS null mice showed an excess of filopodial-like or thin spines lacking heads and a reduction of mature spines having a mushroom shape (Fujii et al., 2005). A third RNA binding protein is mammalian Staufen2. Hippocampal neurons deficient for Staufen2 have reduced dendritic spines and increased filopodia, which result, in part, from impaired *β-actin* mRNA localization (Goetze et al., 2006). Collectively, these studies demonstrate the role of mRNA regulation by mRNA binding proteins in translation affecting spine development, which likely have important consequences for synaptic plasticity, learning, and memory (Klann and Dever, 2004).

Diversity of Neuronal RNA Granules

There is good evidence that transport of mRNAs and translational regulation might be intimately coupled. In neurons, it is generally believed that the transported mRNAs are translationally repressed during transport. New and exciting evidence is now emerging that indicates that translation is activated by specific synaptic inputs (Hüttelmaier et al., 2005; Ashraf et al., 2006; Schratt et al., 2006). The underlying molecular mechanisms of this translational silencing, however, are mainly unknown. There are several interesting scenarios for how this might be achieved in neurons. First, a fair number of translational repressor molecules have been identified that might be responsible for this translational regulation (Klann and Dever, 2004). With the identification of stress granules (SGs) (Table 1), another type of RNA granule (Anderson and Kedersha, 2006), another scenario might be envisioned in dendrites of mature neurons. SGs have been recently proposed to recruit specific mRNA transcripts, thereby regulating their stability and translation (Anderson and Kedersha, 2006). Staufen proteins, established markers for RNA transport in neurons, as well as the mammalian Pumilio2 protein, can be transferred from neuronal RNA granules into dendritic SGs in response to stress (Vessey et al., 2006, and references therein). This is also true for FMRP, as shown by Kim and colleagues, to move out of polyribosomes and into SGs upon oxidative stress (Zalfa et al., 2006, and reference therein). To our knowledge, the vast majority of RNA binding proteins, such as Pumilio2, Barentsz, FMRP, Staufen proteins, and PABP, are sequestered into SGs upon stress. Future work will therefore have to unravel whether the sequestration of mRNAs to SGs may have a function in translational regulation in neurons under physiological conditions. Furthermore, one would like to understand whether transport RNPs might be remodeled into SGs upon stress induction or whether they are distinct structures with separate functions. It will also be interesting to see whether SGs are relatively fixed in their subcellular localization as suggested by Anderson and Kedersha (2006), or alternatively, whether SGs are more mobile than previously anticipated.

Another interesting avenue is the role of processing bodies (P bodies) (Table 1), yet another type of RNA granule (Anderson and Kedersha, 2006) in dendrites of polarized neurons. P bodies contain components of the 5′-3′ mRNA decay machinery, the nonsense-mediated decay pathway, and, most notably, factors involved in small RNA-guided gene silencing. miRNA-guided translational silencing as well as siRNA-guided RNA degradation occur in P bodies. Recent work showed that the sequestration of mRNAs to P bodies may prevent their translation (Brengues et al., 2005). Upon specific signals, such repressed mRNAs can even be released into the cytoplasm for further translation. This is of particular relevance in that miR-134 was recently found in dendritic RNA granules together with its target mRNA, encoding Lim protein kinase 1 (Limk1) (Schratt et al., 2006). miR-134 appears to inhibit the translation of Limk1 at the synapse and causes a reduction in the size of dendritic spines. Interestingly, exposure of neurons to BDNF relieves the inhibition of *Limk1* translation. It is currently unknown whether the observed *Limk1* mRNA and miR-134-positive granules represent transport RNPs or rather another type of neuronal RNA granule, such as P bodies. Therefore, several possible mechanisms can be envisioned. First, the dendritically localized *Limk1* mRNA assembles together with miR-134 into transport RNPs, thereby translationally silencing the mRNA during transport. This may require that components of the RISC complex, such as dicer or argonaute proteins, be present in these transport RNPs as well. Alternatively, *Limk* mRNA could be transported via transport RNPs into dendrites where, subsequently, additional components of the RISC complex are acquired, converting the transport RNPs into P bodies. Recent work has shown that key components of P bodies, such as Dcp1a and Lsm1, exist in dendrites of mature hippocampal neurons (Vessey et al., 2006). It will be exciting to find out whether SGs and P bodies are structures that show motility and whether these RNA granules form in the cell body and are then transported into dendrites, or, alternatively, whether they form in dendrites near synapses. Although the functional significance of P bodies in dendrites and near synapses is still unknown, there is evidence suggesting that P bodies might be involved in regulated, local degradation of certain mRNAs in dendrites such as *GluR1/2* mRNA (Grooms et al., 2006). Further work will therefore have to define the various functions of P bodies at the synapse.

What could be the function(s) of the RISC complex at synapses? At least one RISC factor, the helicase Armitage (Cook et al., 2004), is localized to synapses and degraded by the proteasome in response to neural activity (Ashraf et al., 2006). These investigators proposed the exciting new model that during the formation of long-term memory, Armitage becomes degraded by the proteasome, thereby allowing synaptic protein synthesis and mRNA transport from miRNA-dependent suppression.

In conclusion, recent work has convincingly demonstrated that neuronal RNA granules are much more

heterogeneous in composition and therefore possibly also in function than previously anticipated. Neuronal RNA granules may represent multifunctional molecular machines that are complex, dynamic structures exerting different intracellular functions. Transport RNPs achieve RNA localization and thus warrant precise translational control during transport. An important direction for future work will be to further identify which mRNA binding proteins are essential for mRNA transport, some of them possibly serving as a direct adaptor with subunits of molecular motors. We also need to identify whether there are different types of transport RNPs, harboring different mRNAs and associated binding proteins, which may be developmentally regulated and respond to distinct physiological signals. The presence of SGs in dendrites near synapses suggests that mRNAs may be temporarily stalled for translation upon cellular stress. Whether SGs play a role in translational silencing under physiological conditions remains to be shown. Finally, the discovery of P bodies and microRNAs in dendrites and near synapses immediately suggests a novel and exciting new mechanism for translational silencing and even mRNA degradation. Taken together, future work will have to unravel the precise contributions of these types of neuronal granules to RNA localization, microRNA-mediated translational silencing, and mRNA degradation at the synapse. An important challenge will also be to extrapolate which features of RNA granules and their regulation, observed so far in cultured neurons, will play important roles in vivo. Since neuronal activity and synaptic signaling are known to affect mRNA transport and translation in vivo, the likelihood that the RNA granules here play critical and diverse roles is very strong. Based on the presented findings, we speculate that all of these mechanisms surrounding RNA granules allow for synapse-specific modifications, thereby yielding molecular, structural, and functional reorganization of individual synapses that occur during neuronal development and synaptic plasticity, processes which may go awry in neurological diseases such as fragile X syndrome.

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Selected Reading

Ainger, K., Avossa, D., Morgan, F., Hill, S.J., Barry, C., Barbarese, E., and Carson, J.H. (1993). J. Cell Biol. *123*, 431–441.

Anderson, P., and Kedersha, N. (2006). J. Cell Biol. *172*, 803–808.

Antar, L.N., Afroz, R., Dictenberg, J.B., Carroll, R.C., and Bassell, G.J. (2004). J. Neurosci. *24*, 2648–2655.

Ashraf, S.I., McLoon, A.L., Sclarsic, S.M., and Kunes, S. (2006). Cell *124*, 191–205.

Brengues, M., Teixeira, D., and Parker, R. (2005). Science *310*, 486–489.

Cook, H.A., Koppetsch, B.S., Wu, J., and Theurkauf, W.E. (2004). Cell *116*, 817–829.

Elvira, G., Wasiak, S., Blandford, V., Tong, X.K., Serrano, A., Fan, X., del Rayo Sanchez-Carbente, M., Servant, F., Bell, A.W., Boismenu, D., et al. (2006). Mol. Cell. Proteomics *5*, 635–651.

Eom, T., Antar, L.N., Singer, R.H., and Bassell, G.J. (2003). J. Neurosci. *23*, 10433–10444.

Fujii, R., Okabe, S., Urushido, T., Inoue, K., Yoshimura, A., Tachibana, T., Nishikawa, T., Hicks, G.G., and Takumi, T. (2005). Curr. Biol. *15*, 587–593.

Goetze, B., Tuebing, F., Xie, Y., Dorostkar, M.M., Thomas, S., Pehl, U., Boehm, S., Macchi, P., and Kiebler, M.A. (2006). J. Cell Biol. *172*, 221–231.

Grooms, S.Y., Noh, K.M., Regis, R., Bryan, M.K., Bassell, G.J., Carroll, R.C., and Zukin, R.S. (2006). J. Neurosci. *26*, 8339–8351.

Huang, Y.S., Carson, J.H., Barbarese, E., and Richter, J.D. (2003). Genes Dev. *17*, 638–653.

Hüttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., Bassell, G.J., Condeelis, J., and Singer, R.H. (2005). Nature *438*, 512–515.

Kanai, Y., Dohmae, N., and Hirokawa, N. (2004). Neuron *43*, 513–525.

Kiebler, M.A., and DesGroseillers, L. (2000). Neuron *25*, 19–28.

Klann, E., and Dever, T.E. (2004). Nat. Rev. Neurosci. *5*, 931–942.

Knowles, R.B., Sabry, J.H., Martone, M.E., Deerinck, T.F., Ellisman, M.H., Bassell, G.J., and Kosik, K.S. (1996). J. Neurosci. *16*, 7812–7820.

Köhrmann, M., Luo, M., Kaether, C., DesGroseillers, L., Dotti, C.G., and Kiebler, M.A. (1999). Mol. Biol. Cell *10*, 2945–2953.

Krichevsky, A.M., and Kosik, K.S. (2001). Neuron *32*, 683–696.

Mallardo, M., Deitinghoff, A., Müller, J., Goetze, B., Macchi, P., Peters, C., and Kiebler, M.A. (2003). Proc. Natl. Acad. Sci. USA *100*, 2100–2105.

Monani, U. (2005). Neuron *48*, 885–896.

Rook, M.S., Lu, M., and Kosik, K.S. (2000). J. Neurosci. *20*, 6385–6393.

Schmid, M., Jaedicke, A., Gu, T.G., and Jansen, R.P. (2006). Curr. Biol. *16*, 1538–1543.

Schratt, G., Tuebing, F., Nigh, E.A., Kane, C., Sabatini, M.W., Kiebler, M.A., and Greenberg, M.E. (2006). Nature *439*, 283–289.

St Johnston, D. (2005). Nat. Rev. Mol. Cell Biol. *6*, 363–375.

Tang, S.J., Meulemans, D., Vazquez, L., Colaco, N., and Schuman, E. (2001). Neuron *32*, 463–475.

Vessey, J.P., Vaccani, A., Xie, Y., Dahm, R., Karra, D., Kiebler, M.A., and Macchi, P. (2006). J. Neurosci. *26*, 6496–6508.

Wang, H., Iacoangeli, A., Lin, D., Williams, K., Denman, R.B., Hellen, C.U., and Tiedge, H. (2005). J. Cell Biol. *171*, 811–821.

Wilhelm, J.E., and Vale, R.D. (1993). J. Cell Biol. *123*, 269–274.

Willis, D., Li, K.W., Zheng, J.Q., Chang, J.H., Smit, A., Kelly, T., Merianda, T.T., Sylvester, J., van Minnen, J., and Twiss, J.L. (2005). J. Neurosci. *25*, 778–791.

Zalfa, F., Achsel, T., and Bagni, C. (2006). Curr. Opin. Neurobiol. *16*, 265–269.

Zhang, H.L., Eom, T., Oleynikov, Y., Shenoy, S.M., Liebelt, D.A., Dictenberg, J.B., Singer, R.H., and Bassell, G.J. (2001). Neuron *31*, 261–275.

Zhang, H.L., Xing, L., Rossoll, W.M., Wichterle, H., Singer, R.H., and Bassell, G.J. (2006). J. Neurosci. *26*, 8622–8632.