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Focus Review

Mechanisms of dendritic mRNA transport and its role in synaptic tagging

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The localization of RNAs critically contributes to many important cellular processes in an organism, such as the establishment of polarity, asymmetric division and migration during development. Moreover, in the central nervous system, the local translation of mRNAs is thought to induce plastic changes that occur at synapses triggered by learning and memory. Here, we will critically review the physiological functions of well-established dendritically localized mRNAs and their associated factors, which together form ribonucleoprotein particles (RNPs). Second, we will discuss the life of a localized transcript from transcription in the nucleus to translation at the synapse and introduce the concept of the '*RNA signature*' that is characteristic for each transcript. Finally, we present the '*sushi belt model*' of how localized RNAs within neuronal RNPs may dynamically patrol multiple synapses rather than being anchored at a single synapse. This new model integrates our current understanding of synaptic function ranging from synaptic tagging and capture to functional and structural reorganization of the synapse upon learning and memory.

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Introduction

Memories are an essential part of our lives: some memories persist indefinitely whereas others gradually fade over time. Key to understanding these crucial differences is the identification of the molecular and cellular mechanisms underlying this process. It has been proposed that the physical substrate of 'engrams' or 'traces' for long-term memory (LTM) is the remarkable ability of neurons to alter the efficacy of individual synapses within a relevant neural network in an experience-dependent manner (Govindarajan *et al*, 2006; Redondo and Morris, 2011). It is now generally accepted that the hippocampus—part of the limbic system in mammals—has an important role in the formation of explicit/declarative memories. The ultimate storage of information, however, does not take place in the hippocampus, but has been proposed to occur in the temporal cortex. The hippocampus acts as a filter for the formation of new memories, and changes at glutamatergic excitatory synapses are key to this process (Redondo and Morris, 2011). In particular, two distinct mechanisms have been pinpointed that are important for this process: (1) the activation of gene expression in the nucleus and (2) local protein synthesis at the synapse (Kandel, 2001). These two events—when acting synergistically—are thought to alter individual synapses functionally as well as structurally.

A typical highly polarized neuron, such as a pyramidal cell in the hippocampus, has a cell body, one extended axon and a distinct, fully differentiated dendritic tree. Translation in neurons primarily occurs in the soma, but it is thought that local protein synthesis at synapses far away from the cell body can be triggered by synaptic activation. Increasing evidence points to the presence of polyribosomes at the synapse allowing local translation of specific transcripts, which critically contributes to synaptic plasticity and memory consolidation (Steward and Levy, 1982; Kiebler and DesGroseillers, 2000). However, the regulated translation of synaptically localized mRNAs in the mature brain is not the only known functional contribution of mRNA localization in the central nervous system. Local translation of mRNAs in immature neurites, for example in axonal growth cones, critically contributes to the ability of neurons to respond to environmental cues and to guide its axon to the final destination in the brain (Lin and Holt, 2007).

In order to translate individual transcripts at activated synapses, the mRNAs need to be first selectively transported to these sites. Recently, a number of mechanisms have described how mRNAs can be localized in other experimental systems (from *Drosophila* to mammalian cells) ranging from active transport by motor proteins along the cytoskeleton to diffusion and trapping by a localized anchor as well as local protection from degradation (St Johnston, 2005; Palacios, 2007). In this review, we will focus on the different steps of mRNA localization describing how this process is currently mechanistically envisioned including (1) the presence of *cis*-acting localization elements (LEs) or zipcodes generally located in the 3′-untranslated region (3′-UTR) of localized transcripts, (2) the recognition of these signals by *trans*-acting RNA-binding proteins (RBPs), (3) the assembly of RBPs and their cargo RNAs into transport ribonucleoprotein particles (RNPs) as a functional complex, (4) the

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translocation of transport RNPs along the microtubule (MT) cytoskeleton to their final destination at synapses in a translationally repressed state, (5) the anchoring of these particles at or underneath activated synapses in a translationally repressed state and finally (6) the activation of translation of the localized mRNAs.

While mRNA transport is important in many systems, here we will focus on dendritic mRNA localization, its underlying mechanisms and relationship to synaptic function. We will outline the latest experimental data, demonstrating that RBPs not only have important roles for synapse formation as well as morphogenesis, but also in synaptic plasticity. Finally, we will introduce a simple reductionist model termed 'synaptic sushi belt', which unifies cell body-initiated gene expression, dendritic mRNA localization and local protein synthesis at individual synapses with experience-dependent functional and structural remodelling of synapses during LTM.

mRNA localization in nerve cells

Regulated, localized mRNA translation is especially important in highly polarized cells such as differentiated neurons that may have up to 10 000 dendritic spines—the postsynaptic compartment of a synapse—and at least as many distinct excitatory inputs. It is important to note that the main pathway for protein delivery to synapses—be it the presynaptic or postsynaptic compartment—is via synthesis in the cell body and subsequently transport to either axons or dendrites (reviewed in Kennedy and Ehlers, 2006). However, mRNA localization also occurs during development in axonal growth cones of immature neurons as well as in dendrites of fully mature and polarized neurons. In the brain, mRNA localization is not only restricted to neurons, but also occurs in another type of nerve cell, the oligodendrocytes. Here, the mRNA encoding myelin basic protein (MBP) is selectively delivered into a distinct biochemical compartment in distal processes where myelination occurs (Ainger *et al*, 1993). It is becoming increasingly clear that local translation within the axon critically contributes to axon guidance. This topic has been recently covered in a series of reviews (Hengst and Jaffrey, 2007; Lin and Holt, 2007; Vuppalanchi *et al*, 2009) and therefore will not be discussed here.

In the mature brain, mRNA localization into dendrites of fully polarized neurons serves a distinct function. The presence of a specific set of transcripts and the entire translational machinery at dendritic spines suggests that local translation could be regulated in an activity-dependent manner (Steward and Levy, 1982; reviewed in Sutton and Schuman, 2006). This in turn would allow an individual synapse of a given neuron to modify its function as well as its morphology, thereby providing a mechanism for synaptic plasticity and memory consolidation (Martin and Ephrussi, 2009). The advantages of mRNA localization as a key regulatory mechanism to fine-tune gene expression have been outlined in a series of recent reviews (St Johnston, 2005; Martin and Ephrussi, 2009; Cajigas *et al*, 2010). First, the localization of mRNA rather than its corresponding protein serves a dual function, since it targets the protein directly to the correct intracellular compartment while preventing its expression elsewhere. This is particularly important for those proteins that might be harmful to other parts of the cell, for example MBP in oligodendrocytes, or Tau and MT-associated protein 2 (MAP2) that could bind to all MTs in the cell. Second, it provides a synapse with the unique opportunity to spatially restrict gene expression with high temporal resolution. Therefore, an activated synapse could initiate local protein synthesis that in turn alters its function and morphology in its own microenvironment that is independent of the distant cell body. Third, it is more economic to reuse a given transcript several times for multiple rounds of translation instead of transporting each protein or transcript individually to a distinct synapse.

Interestingly, dendritic protein synthesis appears not to produce general housekeeping proteins, but rather to produce proteins with specialized synaptic functions, for example key kinases (CaMKIIα, PKMζ), cytoskeletal proteins (Arc, MAP2) and neurotransmitter receptors of the AMPA (GluR1 and 2) and NMDA (NR1) families (Bramham and Wells, 2007; Andreassi and Riccio, 2009; Wang *et al*, 2010).

Cis-acting signals or zipcodes

Thirty-one years ago, Blobel (1980) published his Nobel Prize winning signal peptide hypothesis, stating that proteins can be sorted to specific locations inside cells and that the relevant information 'is encoded in discrete 'topogenic' sequences that constitute a permanent or transient part of the polypeptide chain.' Since then, it has become increasingly apparent that other molecules, including mRNAs, contain molecular zipcodes that allow their targeting to distinct subcellular compartments in order to regulate gene expression with high temporal and spatial control. First experimental insights came from *in situ* hybridization (ISH) studies in oocytes, eggs or in asymmetric cells such as fibroblasts, oligodendrocytes and polarized neurons (Steward and Schuman, 2001). Given the unique morphology of highly polarized neurons with dendrites far away from the cell body, the detection of localized messages was straightforward due to physical distance. ISH on brain slices, where the dendrites are largely spatially segregated in the neuropil layer, or on cultured primary neurons is routinely used to assess dendritic mRNA localization. Such experiments allowed Oswald Steward and others to identify dendritically localized mRNAs that still serve as the '*gold standard*', including mRNAs encoding for the activity-regulated cytoskeletal protein (Arc or Arg3.1), the α-subunit of the Ca^{2+}/calmodulin kinase II (CaMKIIα) and MAP2. Whereas Arc mRNA is usually not detected in neurons under basal conditions, since it is a product of an immediate early gene (Plath *et al*, 2006), CaMKIIα and MAP2 mRNAs are routinely detected in dendrites, but in a distinct pattern from each other (Garner *et al*, 1988; Burgin *et al*, 1990).

Since these early analyses, many other mRNAs have now been reported to localize to neuronal dendrites (Bramham and Wells, 2007; Andreassi and Riccio, 2009). Of particular interest for both immature and mature neurons is the β-actin transcript. It is found in neuronal growth cones but also localizes to synapses in fully mature neurons (Tiruchinapalli *et al*, 2003). Another mRNA that has recently received attention in the context of maintaining molecular memory is PKMζ mRNA. It encodes a constitutively active isoform of protein kinase C, which is exclusively expressed in neural tissue (Sacktor, 2011). Its mRNA has been found in dendrites of hippocampal neurons (Muslimov *et al*, 2004). Once

translated locally at activated synapses, high levels of this kinase yield increased numbers of AMPA receptors at post-synaptic sites, potentiating synaptic transmission (Sacktor, 2011).

Many experimental approaches have been applied in neurons to analyse the localization of particular mRNAs in detail. Chimeric constructs consisting of a reporter gene fused to putative LEs were used to visually identify *cis*-acting sequences that direct mRNAs to their destination in dendrites near synapses (Chartrand *et al*, 2001; Martin and Ephrussi, 2009). These experiments convincingly proved the existence of similar 'topogenic' sequences that Blobel (1980) discovered in proteins. These *cis*-acting elements are now commonly referred to as 'LEs' or 'zipcodes' and are often located in the 3′-UTR of the transcript. However, in contrast to the protein zipcodes, these elements can be very heterogeneous in size and structure. They range from 5–6 nts up to very complex secondary structures that can be as much as 1 kb or more in length. An example of a short, well-defined LE is found in MBP mRNA. It contains two partially overlapping 11 nt *cis*-acting RNA sequences in its 3′-UTR that have since been termed the heterogeneous nuclear ribonucleoprotein (hnRNP) A2 response element (A2RE) as they are recognized by hnRNP A2 (Gao *et al*, 2008). The more complex LEs often contain one or several distinct stem loops and are referred to as RNA folds. Because of the complexity of RNA secondary structure, it is challenging to delineate these localization sequences and deduce a clear consensus, either at the level of sequence or structure. In *Drosophila*, Bullock *et al* (2010) were able to identify the first conserved LEs in developmentally important transcripts. These in turn recruit two-core components of a selective dynein motor complex, Egalitarian (Egl) and Bicaudal D (BicD), driving transcript localization in a variety of tissues.

A recent comparison of mRNA localization in oligodendrocytes and neurons revealed that three dendritically localized mRNAs, CaMKIIα, neurogranin and Arc, appear to assemble into the same hnRNP A2 granules. They are targeted by the same A2RE, which also mediates targeting of MBP RNA by the hnRNP A2 pathway in oligodendrocytes (Gao *et al*, 2008). It is tempting to speculate that such conserved signals are recognized by a common group of conserved RBPs that decipher a complex 'RNA signature' on a given transcript (Schnapp, 1999; Bullock and Ish-Horowicz, 2001; Gao *et al*, 2008).

It is important to note that, aside from LEs, regulatory elements found within both the 5′- and 3′-UTR of transcripts can regulate other stages of posttranscriptional control (Andreassi and Riccio, 2009). Interestingly, in the 5′-UTR they appear to be primarily involved in translational control, whereas those in the 3′-UTR can affect various stages, including quality control, nuclear export, localization in the cytoplasm, trafficking to specific intracellular compartments, translational control and mRNA stability (Moore, 2005). Also important to note is that 3′-UTRs often contain binding sites for miRNAs that can trigger the translation repression and/or degradation of an mRNA via the RNA interference (RNAi) pathway. Several studies have reported the presence of miRNAs at the synapse, suggesting that the RNAi pathway locally contributes to synaptic function (Kosik, 2006). Furthermore, it has been hypothesized that mRNA binding of certain RBPs, for example Pumilio2, GW182, Ago and HuR proteins, might either positively or negatively affect miRNA binding and subsequent function (Filipowicz *et al*, 2008; Jacobsen *et al*, 2010).

It is likely that localized transcripts contain more than one copy of a dendritic LE as well as combinations of different zipcodes mediating distinct steps in localization. Complexity can be further increased if these signals (partially) overlap and are recognized by different RBPs. For example, it has been surprisingly difficult to distinguish LEs from other elements regulating translational control. Because of the complexity and the variability of the localization sequences identified to date, it has not yet been possible to unambiguously identify LEs by computational prediction or even to deduce common sequence and structural motifs. In conclusion, a large volume of data on putative LEs within localized transcripts has been collected that now needs to be rigorously validated in its physiological context. Ultimately, RNA signatures for each localized transcript have to be determined experimentally.

To this end, the development of new experimental approaches that allow high-resolution imaging of mRNA localization in living neurons will undoubtedly yield new mechanistic insight in the underlying process of mRNA localization. In addition, both loss-of-function as well as gain-of-function experiments must be applied *in vivo* to selectively interfere with either the LEs of the RNA to be studied or the RBP(s) that selectively bind(s) to the LE. Analysis of the process of mRNA localization in neurons would then show whether localization is selectively impaired or whether other aspects of mRNA metabolism are also affected.

We would like to highlight two key studies (Miller *et al*, 2002; Lionnet *et al*, 2011) that investigated the localization of the prominently dendritically localized mRNAs, CaMKIIα and β-actin, in their physiological context at the activated synapse. For CaMKIIα mRNA, there are conflicting studies defining its LE(s). Mori *et al* (2000) identified a 94-nt long element in the 3′-UTR of the CaMKIIα transcript that proved to be sufficient to target a GFP reporter construct to dendrites. They further identified a larger element downstream of the first that exhibited a dominant-negative effect on RNA localization. Using a similar reporter assay, Kindler and coworkers identified a distinct LE in the middle of the 3′-UTR of CaMKIIα mRNA (Blichenberg *et al*, 2001). These experiments clearly demonstrate how difficult it is to interpret the results of deletion and overexpression studies. Mayford and colleagues went on to investigate the *in vivo* role of the CaMKIIα 3′-UTR at the synapse by generating a mutant mouse that was lacking most of the 3′-UTR (Miller *et al*, 2002). However, the 94-nt 'Mori element' was still present. ISH analyses of brains from these mice showed that the mutant CaMKIIα mRNA containing the entire 5′-UTR, coding region and the 'Mori element' failed to localize to dendrites. This confirms *in vivo* that CaMKIIα 3′-UTR is necessary for dendritic targeting and that the Mori element alone is not sufficient. Very recently, Moine and colleagues have described a G-quadruplex RNA structure in the CaMKIIα 3′-UTR that directs the RNA into cortical neurites (Subramanian *et al*, 2011). Consequently, the localization of the CaMKIIα mRNA appears to depend on multiple LEs, and further work is needed to delineate which are indeed necessary and sufficient *in vivo* and which may act synergistically.

Most importantly, late-phase long-term potentiation (L-LTP) was reduced in the 3′-UTR mutant mice, providing the strongest evidence to date for a functional contribution of dendritic mRNA localization to the stabilization of synaptic plasticity and memory consolidation. Furthermore, spatial memory, associative fear conditioning and object recognition memory were also impaired (Miller *et al*, 2002). To date, no similar mutant mice for other well-known dendritically localized transcripts are available. Such mouse models will substantially increase our understanding of mRNA localization in the nervous system and its contributions to synaptic function and learning and memory.

Recently, a novel approach to study the dynamics of mRNA localization *ex vivo* was published by Singer and colleagues (Lionnet *et al*, 2011). This is based on the bacteriophage MS2 imaging system they previously established, where an RNA of interest is tagged with MS2-binding sites (MBS) that are recognized by the MS2 coat protein (MCP), which in turn is fused to a fluorescent protein. The RNA and the MCP are expressed from different plasmids that are cotransfected in the same cell, thus allowing visualization of the overexpressed MCP bound to the MBS on RNA. A careful comparison of the localization pattern of the overexpressed transcript with the endogenous RNA is required to ensure that the insertion of multiple MBS reflects the normal physiological localization. In mammalian cells, this system has previously only been used in transiently transfected cells. Singer and colleagues now applied the system *in vivo* by generating a transgenic mouse where they inserted an MBS cassette into the endogenous β-actin locus, in the 3′-UTR, which still contains its normal LE. Using primary hippocampal neurons derived from these mice, the authors studied the transport of the endogenous β-actin mRNA *ex vivo*. When they coexpressed an MCP-YFP reporter in these neurons, they were able to track single endogenous labelled β-actin mRNA particles on their way into dendrites. It is important to note that the MBS cassette did not interfere with the dendritic targeting of the β-actin transcript. This is a crucial technological advancement, since it now allows the visualization of an endogenously expressed mRNA at normal expression levels in contrast to reporter constructs that often yield overexpression artefacts. Therefore, the β-actin-MBS mouse provides a powerful tool for many future experiments: (1) for live cell imaging of an endogenous localized transcript in brain tissue *in vivo*, for (2) biochemical isolation of β-actin-RNPs in order to unravel its binding partners in the brain and for (3) crossing these mice with other mouse models to assess the roles of other factors involved in mRNA localization (e.g. RBPs—see below) (Lionnet *et al*, 2011).

Trans-acting factors or RBPs

In a simplistic model of mRNA localization, a single LE could be recognized by a single RBP. Up-to-now, only one mRNA appears to fall into this category: the MBP mRNA containing the A2RE, which is specifically recognized by hnRNP A2. It is both necessary and sufficient to direct MBP mRNA towards oligodendrocyte processes (Hoek *et al*, 1998; Munro *et al*, 1999). All other well-characterized mRNAs that localize to dendrites contain LEs that are more complex, often folding into higher-order secondary structures. These mRNAs might contain multiple elements that are (at least) partially redundant or exert related regulatory functions.

Consequently, it is necessary to investigate the *trans*-acting factors that bind the *cis*-acting elements to link an mRNA to the localization machinery and achieve its targeting to the activated synapse. A likely scenario is that RBPs or associated proteins provide the link to the molecular motor involved in this process (Dictenberg *et al*, 2008). There are three possible mechanisms by which RBPs are recruited to regulatory elements that might promote localization (Palacios, 2007): (1) active and direct transport of the transcript via molecular motor proteins and the neuronal cytoskeleton; (2) facilitated diffusion and subsequent trapping of the transcript by a localized anchor and (3) protection from degradation only at the site of localization.

Over the last 20 years, a large number of RBPs have been implicated in mRNA localization in various organisms. Most of these were either identified in genetic screens involved in mRNA localization or in biochemical and proteomic approaches (Kiebler and DesGroseillers, 2000; Bassell and Kelic, 2004; St Johnston, 2005; Bramham and Wells, 2007; Holt and Bullock, 2009; Martin and Ephrussi, 2009). A careful comparison of these factors showed that some of them belong to large, well-conserved families of RBPs (Schnapp, 1999; Bullock and Ish-Horowicz, 2001). RBPs that were characterized for their contribution to dendritic mRNA localization include ZBP1, the Staufen proteins, fragile × mental retardation protein (FMRP), the cytoplasmic polyadenylation element-binding protein (CPEB) and the hnRNP A2 protein. However, very few of the identified RBPs have been assigned specific roles during the multi-step process of mRNA localization (see Figure 1).

ZBP1 protein is one of the best-studied neuronal *trans*-acting factors, with its expression peaking during brain development. In developing neurons, ZBP1 binds to the zipcode of β-actin mRNA in axonal growth cones and critically contributes to growth cone navigation by regulating the stimulus-induced local translation of β-actin (Lin and Holt, 2007). During later development, ZBP1 contributes to proper dendritic branching (Perycz *et al*, 2011). In fully polarized neurons, a ZBP1 β-actin mRNA complex selectively trafficks into dendritic spines upon synaptic stimulation (Tiruchinapalli *et al*, 2003). The essential role of ZBP1 in promoting β-actin localization is the inhibition of β-actin mRNA translation. This is abrogated upon phosphorylation of ZBP1 by Src kinase, resulting in the release of β-actin mRNA from mRNPs and the activation of mRNA at the site of high actin dynamics, for instance exploratory growth cones (Hüttelmaier *et al*, 2005). Interestingly, Bassell and colleagues provided detailed insight into the role of tyrosine phosphorylation of ZBP1 in neurons. They showed that phosphorylation of ZBP1 at Y396 within growth cones regulates local protein synthesis and growth cone turning (Sasaki *et al*, 2010).

The Staufen proteins are some of the best-known proteins involved in mRNA localization in many species. Staufen orthologues have been implicated in mRNA transport in *Drosophila*, the sea mollusc *Aplysia* and vertebrates (St Johnston, 2005). Staufen proteins belong to the family of double-stranded RBPs (St Johnston *et al*, 1992). In mammals, two Staufen proteins exist: a ubiquitously expressed Staufen 1 (Stau1) that has been implicated in mRNA localization and

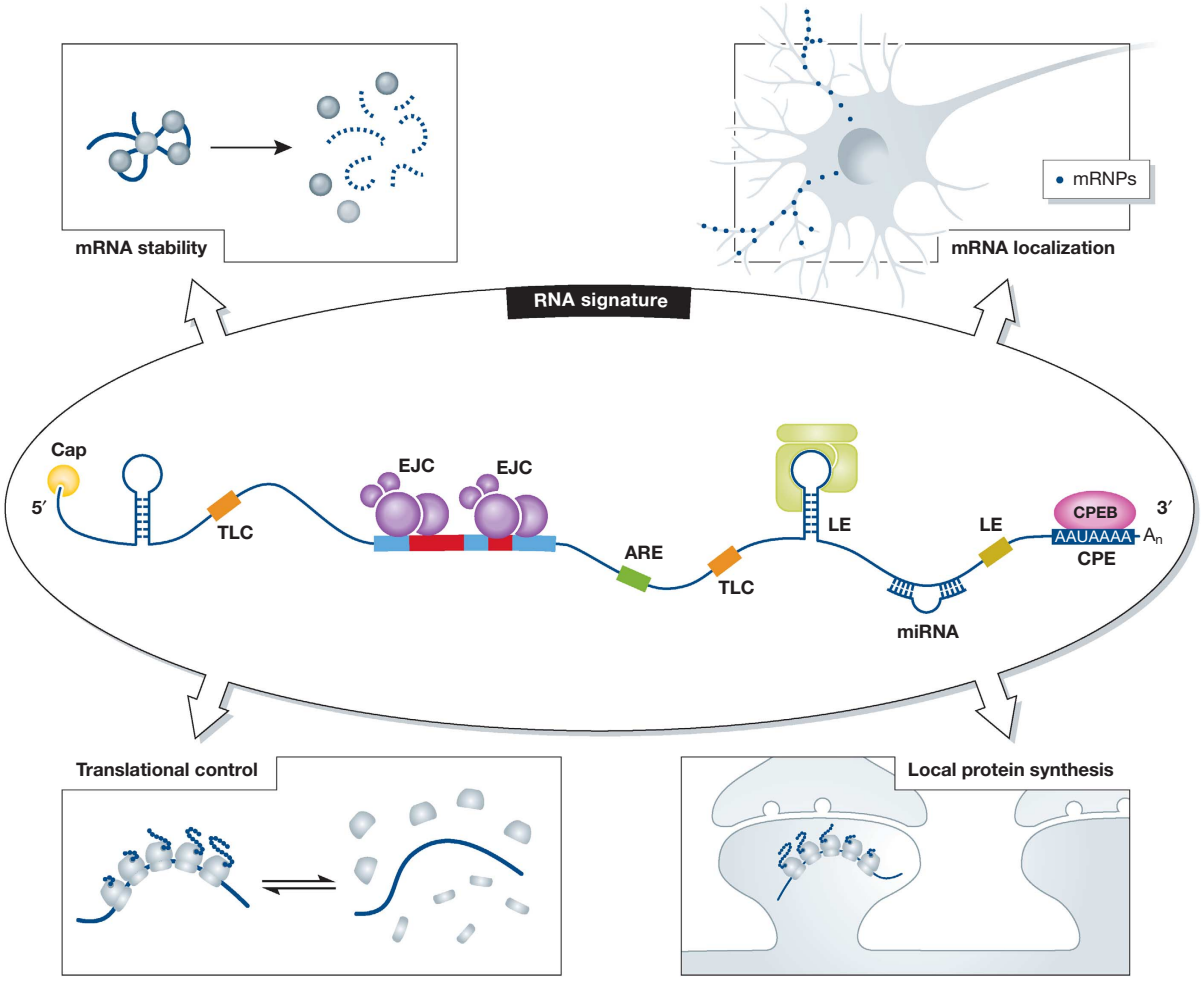

Figure 1 RNA signature. mRNAs—as depicted in the centre of the figure—contain multiple regulatory elements at both the primary sequence and structural levels that are recognized by certain *trans*-acting factors or RBPs. These can bind either single-stranded (simple line) or double-stranded (hairpin structures) RNA. In addition, some of them can also interact with other proteins as shown on the right. Consequently, each transcript has its own unique 'RNA signature', which determines the fate and function of an mRNA including its stability, localization, translational control and whether it undergoes local protein synthesis (functions listed in the outer panels). As every mRNA can contain various combinations of regulatory elements and bind different RBPs, this not only increases the functionality of a transcript, but also offers unique ways to regulate the fate of the RNA. ARE, AU-rich element; Cap, 7-methylguanosine; CPE, cytoplasmic polyadenylation element; CPEB, cytoplasmic polyadenylation element-binding protein; EJC, exon junction complex; LE, localization element; miRNA, microRNA bound to its miRNA-binding site; mRNPs, messenger RNA-containing RNPs; TLC, translation control element.

decay (Vessey *et al*, 2008; Gong and Maquat, 2011) and Staufen 2 (Stau2), which is preferentially expressed in the brain. Because of alternative splicing, four major Stau2 isoforms are expressed in the mammalian brain (Mallardo *et al*, 2003; Monshausen *et al*, 2004). Domain organization of the longest Stau2 isoform, Stau2^{62}, is most similar to *Drosophila* Staufen (Duchaine *et al*, 2002). Interestingly, Stau2^{62} is a nucleocytoplasmic shuttling protein (Macchi *et al*, 2004; Miki *et al*, 2005) and was therefore proposed to transport RNAs from the nucleus into neuronal dendrites, keeping them in a translationally repressed state until synaptic activity relieves the translational block (Goetze *et al*, 2006). The first evidence that Staufen proteins are directly involved in synaptic plasticity came from Lacaille and colleagues. They showed that Stau1 is required for the late phase of LTP (Lebeau *et al*, 2008). In contrast, Stau2 downregulation appears to affect only metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) (Lebeau *et al*, 2011). These studies clearly demonstrate that RBPs, in this case Staufen proteins, critically contribute to synaptic plasticity. Moreover, the two Staufen proteins appear to have specific roles in separate physiological processes, arguing for their distinct functions at the synapse.

Another important *trans*-acting factor involved in dendritic mRNA localization is FMRP, which is abundant in the brain, where it is thought to regulate many different mRNAs (Zalfa *et al*, 2006; Bassell and Warren, 2008). FMRP binds to several localized transcripts, including CaMKIIα, MAP1b, PSD-95 as well as its own mRNA. Recent evidence suggests that FMRP regulates mRNA transport in dendrites (Dictenberg *et al*, 2008). In this study, Bassell and coworkers reported a direct association of FMRP with kinesin, suggesting that FMRP links CaMKIIα mRNA (and other mRNAs) to a molecular motor. In addition, FMRP is one of the best-characterized translational regulators at the synapse. There, it regulates local translation of mRNAs upon the activation of mGluRs. Recent work has shown that the phosphorylation status of FMRP and its interaction with miR-125a controls PSD-95 mRNA translation

at synapses (Muddashetty *et al*, 2011). Under conditions where FMRP is nonfunctional, as in fragile X syndrome, altered dendritic spine morphology, synaptic function and loss of translation-dependent synaptic plasticity can likely be attributed to dysregulated mRNA translation (Bassell and Warren, 2008). Taken together, these studies show that, like ZBP1, FMRP has a dual function in mRNA transport and local translation.

A fourth *trans*-acting factor, lately receiving attention for its role in dendritic mRNA localization and translational control is CPEB (Richter, 2007). In mammals, there are four genes encoding CPEBs: CPEB1 has been shown to bind CPEs, whereas CPEB2–4 might exert other functions (Theis *et al*, 2003; Huang *et al*, 2006). In contrast to FMRP, which is thought to inhibit protein synthesis, CPEB activates translation. In *Aplysia*, a neuron-specific isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation (Si *et al*, 2003). It is of note that several dendritically localized transcripts, among them CaMKIIα mRNA, contain CPEs. Binding of CPEB to these elements within mRNAs was shown to affect their translation. Furthermore, CPEB has been implicated in dendritic targeting (Huang *et al*, 2003). Thus, CPEB appears to be yet another RBP with dual function in mRNA localization in mammalian cells.

This detour into specific examples of RBPs shows what diversity exists in terms of their functional contribution to mRNA localization. Over the last few decades, various domains in RBPs that mediate RNA binding have been identified (Stefl *et al*, 2005; Lunde *et al*, 2007). Some of these bind single-stranded RNA and recognize a primary sequence motif (e.g. hnRNP A2, hnRNP U), whereas others recognize a secondary structure in the RNA (e.g. Staufen proteins, ADAR proteins, Dicer) or both a primary sequence as well a secondary structure (e.g. ADAR proteins, ZBP1) (Stefl *et al*, 2005). Staufen proteins contain multiple copies of dsRBDs, a conserved domain of ~70 amino acids (St Johnston *et al*, 1992). In addition, many of the RBPs also have protein interacting domains and in the case of Staufen proteins, some of the dsRBDs have been shown to function as protein interaction domains independent of RNA binding (Irion *et al*, 2006). Several structures of dsRBDs are available, some complexed with dsRNA, revealing a common αββββα topology where the two α-helices lie on the face of a three-stranded anti-parallel β sheet. Significantly, these structures reveal that dsRBDs interact with the sugar–phosphate backbone of the RNA without any direct base contact, suggesting a lack of sequence specificity (Doyle and Jantsch, 2002). *In vivo* microinjection experiments, however, demonstrated binding specificity for *Drosophila* Staufen (Ferrandon *et al*, 1994).

An increasingly attractive hypothesis is that different dsRBDs cooperate intramolecularly in regulating the specificity of Staufen proteins and its biological activity (Lunde *et al*, 2007). Experiments using other dsRBD-containing proteins lend support to this model. Bass and coworkers studying the human RNA editing enyzme ADAR2 found that the N-terminal dsRBD1 inhibits the association of dsRBD2 and the catalytic domain with a substrate RNA. Only if both dsRBDs bind to the RNA, does the enzyme actively edit the substrate (Macbeth *et al*, 2004). There are also similar examples of intermolecular cooperativity of RBDs and proteins in lower organisms. In *Drosophila*, Egl and BicD proteins increase their affinity for RNA binding when acting together (Dienstbier *et al*, 2009). Other examples of cooperative binding include the yeast She2p and She3p RBPs, which together achieve synergistic cargo binding, yielding dramatically increased specificity for localizing RNAs (Müller *et al*, 2011). Together, these examples demonstrate the importance of both intra- and intermolecular interactions of RBPs with substrates and their contribution to RNP cargo recognition.

To link the activities of these various RBPs back to their targets, a serious effort has been made to identify novel localized mRNAs associating with these *trans*-acting factors—either directly or via intermediates. Advances in systems biology approaches, in particular genome-wide microarray analysis, deep sequencing and CLIP (crosslinking after immunoprecipitation) have led to the identification of many RBP-associated mRNAs, some of which may be dendritically localized and important for synaptic function (Hieronymus and Silver, 2004; Chi *et al*, 2009; Hafner *et al*, 2010; Maher-Laporte and DesGroseillers, 2010). It is important to note, however, that this method is limited to direct RBP–RNA interactions and any complex can be missed. Although experimental validation of localization and function is ultimately required, these new methods provide a valuable resource in order to understand the functional contribution of an RNA and its bound RBP(s) at the synapse. Taken together, this should eventually yield the underlying 'RNA signature' of a given localized mRNA by revealing the various regulatory elements that contribute to localization, transport and RNP formation (see Figure 1).

CLIP has been a particularly informative approach in advancing our understanding of the various aspects of mRNA metabolism (Ule *et al*, 2003). For example, Darnell and colleagues investigated targets of the brain-specific RBPs Nova1 and Nova2, which are involved in the splicing of gephyrin, a protein required for the correct localization of two distinct $GABA_A$ γ2 and GlyRα2 subunits each to the inhibitory synapse (Ule *et al*, 2003). High-throughput sequencing of CLIP data (HITS–CLIP) identified the positions on 3′-UTRs of mRNAs that directly interact with Nova proteins, indicating that these mRNAs might colocalize with Nova at the synapse. Thus, CLIP has the potential to identify RNA LEs. This approach was used to show that Nova proteins bind to the 3′-UTR of the GIRK2 mRNA and that this is required to localize a GIRK2 reporter transcript to neuronal processes (Racca *et al*, 2010).

RNP assembly and transport along the cytoskeleton

We have previously reviewed the discovery of neuronal RNPs, the attempts to purify them biochemically, and their relationship to other types of RNA granules, such as stress granules and processing bodies (Kiebler and Bassell, 2006, see also Anderson and Kedersha, 2006). New technology remains key for successful visualization of these transport RNPs. In the case of MBP mRNA trafficking, Ainger *et al* (1993) used fluorescently labelled MBP mRNA and microinjected it into cultured oligodendrocytes and studied the transport of RNA granules along MTs. In neurons, three different approaches were taken. Initially, Kosik and colleagues used a fluorescent vital RNA dye called SYTO14 to study the transport of endogenous RNA granules into dendrites of

cultured cortical neurons (Knowles *et al*, 1996). Koehrmann *et al* (1999) took advantage of Staufen as a stable marker to track neuronal transport RNPs. A further approach came from the Kosik laboratory (Rook *et al*, 2000) using the MS2 imaging system from Singer and colleagues (Bertrand *et al*, 1998). Here, Rook *et al* generated a reporter system to track the movement of CaMKIIα mRNA-containing granules in dendrites of hippocampal neurons (see also Kanai *et al*, 2004; Dictenberg *et al*, 2008). A similar approach was taken by Brechbiel and Gavis (2008) who tracked the movement of nanos mRNA that localizes in *Drosophila* larval peripheral neurons. They could show that localization and translational control of nanos are both essential for larval sensory neuron morphogenesis. Taken together, these initial studies showed that neuronal RNPs are very dynamic structures in dendrites and very heterogeneous in size and in their composition (Kiebler and Bassell, 2006; Bramham and Wells, 2007).

How can we envision the assembly of such neuronal RNPs? The various regulatory signals in a dendritically localized transcript might serve as '*recognition platforms*' for the recruitment of *trans*-acting factors or RBPs (Andreassi and Riccio, 2009). The first contact of RBPs to nascent transcripts occurs in the nucleus (Dreyfuss *et al*, 2002; Moore, 2005) and pre-mRNA processing in the nucleus is required for the assembly of appropriate RNP and thus for mRNA localization. In addition, the existence of alternative polyadenylation sites can yield different RNA isoforms that might be targeted differently. Thus, the nuclear history of an mRNA is critical to both RNP formation and its subsequent cytoplasmic localization (Giorgi and Moore, 2007). There is evidence that RNPs undergo extensive remodelling from the beginning of assembly in the nucleus up to the point of arrival at their synaptic destination (see below).

Several examples demonstrate how nuclear events during the maturation of an mRNA influence this process, with the role of the ZBPs and exon junction complex (EJC) receiving much attention in recent years. As pointed out earlier, ZBP1 is part of a larger family of RBPs that binds to the zipcode of β-actin mRNA and other mRNAs. ZBP2 and its orthologues (KHSRP in human and MARTA1 in rat) are predominantly nuclear proteins and have all been implicated in mRNA localization. Chicken ZBP2 has recently been shown to assist ZBP1 in β-actin mRNA binding (Pan *et al*, 2007). These findings have strong implications for our understanding of mRNA localization in mammalian cells. First, ZBP2 appears to contribute to cytoplasmic mRNA localization by forming a competent RNP in the nucleus. Second, it is an excellent example of how cooperative binding between different RBPs and its cargo mRNA might control the assembly and maturation of RNPs (see above). Third, the localization of mRNAs is essentially linked to the spatio-temporal control of protein synthesis, which in turn is regulated by synaptic signal cascades.

Another key example of nuclear history affecting RNP formation is the EJC. This multi-protein complex contains a core of four proteins comprising eIF4AIII, Barentsz/MLN51, Magoh and Y14. It is deposited upstream of every exon junction on spliced mRNA (Moore, 2005). Together with an mRNA, the EJC is exported to the cytoplasm where it is removed during the first 'pioneer round' of translation. Until its removal, the EJC influences the fate of an mRNA in several ways. When bound to the coding region of a transcript, it can enhance translation by recruiting polysomes, whereas if bound to the 3′-UTR, or after a premature stop codon, it has the opposite effect—triggering nonsense-mediated decay (NMD). However, the EJC has also been demonstrated to be necessary for mRNA localization and RNP formation. In *Drosophila*, all four core EJC components are required for *oskar* mRNA localization to the posterior pole of developing germ cells (Le Hir *et al*, 2001; Hachet and Ephrussi, 2004). Moreover, experiments expressing an *oskar* mRNA from a transgene showed that at least one intron was required for its proper localization (Hachet and Ephrussi, 2004). Recently other studies, including those in mammals, have confirmed the involvement of the EJC in mRNA localization and RNP formation. Moore and colleagues demonstrated that eIF4AIII is associated with neuronal RNPs, colocalizing with Stau1 and FMRP in dendrites (Giorgi *et al*, 2007). Its depletion increases synaptic strength and abundance of GLUR1 AMPA receptors at synapses. Interestingly, loss of eIF4AIII increased the expression of Arc, since Arc mRNA contains a conserved intron in its 3′-UTR that targets it for NMD after the pioneer round of translation. Therefore, as well as influencing the localization of mRNAs including translational control (Le Hir and Seraphin, 2008), the EJC has a role in quality control for certain localized messages.

The most convincing study to date supporting the view of sequential recruitment of RBPs to a transcript comes from experiments in *Xenopus* (Kress *et al*, 2004). The authors microinjected either Vg1 or VegT RNAs, two localized transcripts, into oocytes and performed biochemical fractionation and immunoprecipitations in order to investigate the binding of four RBPs to these transcripts. Two RBPs, hnRNP I and the *Xenopus laevis* ZBP1 homologue Vera/Vg1RBP, bound these RNAs in the nucleus. However, Prrp and Staufen both associated later in the cytoplasm arguing that both proteins might be involved in RNP remodelling.

Further remodelling of RNPs must eventually include the recruitment of molecular motors to trigger cytoplasmic mRNA transport to its synaptic destination. However, little is known on the molecular link between a transcript and its localization machinery. Possibly the best-understood link was unravelled by Bullock and colleagues in *Drosophila* where the molecular mechanism of RNP assembly and the recruitment of the molecular motor Dynein was described in great detail (Dienstbier *et al*, 2009). Less is known in neurons with the best evidence to date by Dictenberg *et al* (2008), who suggested a direct link between Kinesin heavy chain (KIF5) and FMRP. In addition, Kanai *et al* (2004) were able to interfere with dendritic CaMKIIα mRNA localization using a dominant-negative KIF5 mutant. Finally, recent evidence by Kandel and coworkers suggests that specific isoforms of Kinesin heavy chain are upregulated upon induction of long-term facilitation in *Aplysia* neurons (Puthanveettil *et al*, 2008), which then carry specific cargo proteins, for example neurexin, neuroligin, piccolo and bassoon, to the synapse. This activity-dependent regulation of Kinesin may therefore have implications for mRNA transport to the synapse.

There is yet another important implication for the transport of RNPs along the neuronal cytoskeleton. For a long time, it was assumed that there are only actin filaments, but no MTs in dendritic spines. Myosin Va was shown to facilitate the accumulation of RNPs, including the RBP TLS and its

cargo mRNA Nd1-L, an actin stabilizer, in dendritic spines (Yoshimura *et al*, 2006). Recent evidence, however, showed that dynamic MTs can actually enter dendritic spines (Hu *et al*, 2008; Jaworski *et al*, 2009). These findings suggest that there are two options for synaptic delivery of RNPs. In addition to the conventional transport along dendritic MTs followed by MyoVa-dependent transport along actin filaments into dendritic spines, dynamic MTs represent the second option. These could preferentially extend into active synapses offering the unique possibility of selective transport and/or patrolling (sushi belt model, see below) of RNPs to these synapses that undergo activity-dependent modulation.

What is the molecular nature of these neuronal RNPs? While there is growing evidence on the molecular composition of proteins present in these neuronal RNPs (reviewed in Sossin and DesGroseillers, 2006), much less is known about the RNA composition of endogenous neuronal RNPs. In this context, we would like to address two key questions. First, how many copies of the same RNA might be present in individual RNPs? In *Drosophila*, it is known that oligomeric RNAs can assemble into RNPs, indicating that multiple copies can be cotransported (Hachet and Ephrussi, 2004). However, evidence in other systems is lacking. Second, do RNA transport particles contain several different RNAs? In budding yeast, Jansen and coworkers have provided compelling evidence that multiple RNAs known to localize via the Myo4p/She2p/She3p complex are found in the same particles in live cells (Lange *et al*, 2008). Furthermore, Carson and coworkers showed that three localized mRNAs all containing A2REs coassemble into the same RNA granules for hnRNP A2-dependent delivery to dendrites (Gao *et al*, 2008). One established method in the field has been to inject labelled RNAs into mammalian cells including neurons. In those experiments, dendritic targeting of mRNAs did not require a nuclear event (Tübing *et al*, 2010). Furthermore, this method allowed for coinjection and simultaneous visualization of two RNAs and revealed that dendritically localized transcripts, for example CaMKIIα and MAP2 mRNAs, appear to be differentially sorted into dendritic RNPs (Tübing *et al*, 2010), indicating that different dendritic trafficking pathways exist. Those transcripts belonging to the same pathway are coassembled into the same RNPs, for example the hnRNP A2 pathway, whereas transcripts belonging to distinct trafficking pathways will be differentially sorted. We recently addressed this by applying double fluorescent ISH to demonstrate that MAP2, CaMKIIα and β-actin mRNAs localize in distinct RNPs in dendrites of hippocampal neurons (Mikl *et al*, 2011). This suggests that independent localization of these mRNAs might represent yet another mechanism to allow distinct regulation at individual synapses. Clearly, further work is necessary to identify different dendritic trafficking pathways and group the various localized transcripts accordingly based on an RNA transport assay in living neurons.

mRNA localization and translational regulation

Even 15 years ago, Tiedge and Brosius (1996) reported that many components of the translational machinery including ribosomes, tRNAs, as well as translation initiation and elongation factors were found in dendrites of hippocampal neurons. Krichevsky and Kosik (2001) then went on to isolate a very heavy fraction of ribosomal clusters that also contained Stau1 and localized transcripts such as CaMKIIα mRNA. As depolarization with KCl released CaMKIIα mRNA from this heavy fraction into polysomes, the authors concluded that RNA granules are a local storage compartment for translationally silent mRNAs. As outlined above, Hüttelmaier *et al* (2005) provided convincing evidence that ZBP1 not only regulates β-actin mRNA localization but also translational repression during transport. This can be relieved by tyrosine phosphorylation of ZBP1 via Src kinase, which has been shown *in vitro* to affect the ability of ZBP1 to bind to the β-actin transcript. Such mechanisms had been previously described for other RBPs (Ostareck-Lederer *et al*, 2002). This led to the formulation of a new hypothesis: localized transcripts travel within RNPs to their final synaptic destination in a translationally repressed state due to the presence of various binding partners acting as translational regulators. Once an individual synapse is activated, RNPs partially disassemble and translational repression is relieved locally (see Dahm and Kiebler, 2005; Meignin and Davis, 2010). In addition, P-body components were suggested to be present in neuronal transport RNPs. Furthermore, Richter and coworkers showed that CaMKIIα mRNA undergoes polyadenylation and translational activation in response to visual experience (Wu *et al*, 1998). These findings prompted the authors to propose that CPEB represents an important translational regulator at the synapse due to its capacity to regulate poly(A) tail length of synaptically localized transcripts. It is of particular interest that a neuron-specific form of CPEB identified by Kandel and colleagues has prion-like properties, providing a fascinating novel mechanism for activity-dependent changes in long-term facilitation to persist for a long time (Si *et al*, 2010). Taken together, it will be very interesting to see which of the many translational regulators implicated in dendritic mRNA localization are necessary and sufficient to repress translation during transport. Finally, increasing evidence suggests that RNA binding defects in translational regulators often cause diseases (Hieronymus and Silver, 2004). Therefore, we expect that the systematic analysis of mRNP biology in health and disease will identify many more disease genes involved in mRNA localization and translational control.

Anchoring of transcripts at the synapse

One of the least understood steps in mRNA localization is whether and how transcripts are anchored at synapses after transport. Most of the evidence for our understanding on anchoring comes from other experimental systems. For example, it has been suggested that actin or actin-binding proteins are involved in this step (Delanoue and Davis, 2005; Martin and Ephrussi, 2009; Meignin and Davis, 2010). Interestingly, even a molecular motor, the MT minus-end directed Dynein, has been proposed as a static anchor that remains with its cargo RNA at its final destination in *Drosophila* blastoderm embryos (Delanoue and Davis, 2005). Even though transcript anchoring at synapses is poorly understood, Bramham and Wells (2007) recently proposed an interesting reciprocal interaction model between actin dynamics and dendritic protein synthesis that contributes to synaptic plasticity. In this model, the actin cytoskeleton—in

or underneath dendritic spines—might influence local translation. In addition, a feedback loop was proposed by the authors in which the local actin cytoskeleton is regulated by newly synthesized proteins, for example β-actin, Arc or possibly LimK1.

However, there might be alternative views to static anchoring. As we will outline below, we propose that neuronal RNPs could routinely patrol a group of synapses in dendrites. In support of this, there is recent evidence from *Drosophila* by the St Johnston laboratory that there is multi-directional trafficking of *oskar* mRNA along a weakly polarized cytoskeleton, with only a slight bias to the posterior pole of the oocytes (Zimyanin *et al*, 2008). It is therefore possible that such a bias might also exist for localized messages into dendrites. Bidirectional transport of mRNAs within dendrites has been observed in many laboratories (Knowles *et al*, 1996; Koehrmann *et al*, 1999; Dynes and Steward, 2007; Dictenberg *et al*, 2008; reviewed in Kiebler and Bassell, 2006). However, whether the cytoskeleton exhibits a bias as it does in the *Drosophila* oocyte remains unclear.

Synaptic tagging and capture

The local cytoskeleton within dendritic spines is important for activity-dependent changes occurring at individual synapses during learning and memory (Govindarajan *et al*, 2006; Sutton and Schuman, 2006; Bramham and Wells, 2007; Costa-Mattioli *et al*, 2009; Ramachandran and Frey, 2009; Richter and Klann, 2009). In this context, long-lasting synaptic changes were shown to require nuclear transcription followed by delivery of newly synthesized proteins to the synapse to yield synaptic remodelling. However, there are conflicting data on whether transcription is immediately required after training (Kandel, 2001) or alternatively, only after 4–8 h (Frey and Frey, 2008). In addition, the activated synapse requires a local signal that not only marks it for further remodelling but also allows it to capture proteins or mRNAs for protein-synthesis-dependent LTP or LTD and memory formation *in vivo*. This process has been termed synaptic tagging (Frey and Morris, 1997). These findings indicate a tight and extensive dialogue between the synapse and the nucleus in both directions (Kandel, 2001). Furthermore, this hypothesis predicts that newly synthesized proteins delivered by nondirected transport from the cell body must be captured locally at the activated synapse in order to function in an input-specific manner (Okada *et al*, 2009).

There is much debate, however, on the physical nature of the synaptic tag(s), since most likely it is more than one molecule and it may differ in distinct regions of the brain. Some possible mechanisms to create synaptic tags are post-translational modifications of pre-existing proteins at the activated synapse; a prion-like switch in protein conformation, for example CPEB; or alternatively, initial local translation might generate a platform for further translation. In other words, this may create the molecular basis to tag a synapse. For example, in apical CA1 dendrites, local translation may generate or activate tag molecules like CaMKII (Sajikumar *et al*, 2007). Subsequently, these tagged synapses could capture plasticity-related effector proteins synthesized by either another set of local mRNAs or newly synthesized proteins from the cell body (e.g. PKMζ). Such a mechanism would allow an activated synapse to be tagged for the consolidation of protein-synthesis-dependent LTP and LTM (Bramham and Wells, 2007) and could explain how mRNA targeting and local protein synthesis actually contributes to synaptic tagging (Frey and Frey, 2008).

The 'sushi belt' model

We propose uniting the contribution of mRNA localization and local protein synthesis with the idea of synaptic tagging and capture and the subsequent experience-dependent functional and structural remodelling of individual synapses. As discussed above, one of the least well-understood aspects in dendritic mRNA localization is the anchoring of localized transcripts at the activated synapse. Many laboratories have observed bidirectional trafficking of RNPs within dendrites near synapses as described above. We therefore postulate that there may not be permanent anchoring of RNPs at the synapse, but instead a constant bidirectional transport in dendrites of mature neurons (Figure 2). Thus, neuronal transport RNPs could routinely patrol a group of synapses in dendrites—like a conveyor belt in a Japanese sushi restaurant serving all potential customers. In the restaurant, the customer can simply take sushi from the conveyor belt; the hungrier the customer is, the more frequently they will take it. At the synaptic level, it may not be possible to 'take' RNPs unless the synapse has been previously activated. Thus, by analogy, a synapse that has recently undergone activity-dependent tagging represents a '*hungry synapse*' that takes sushi off the belt—in other words, recruits a neuronal RNP.

One possible consequence of synapse tagging could be that it attracts dynamic MTs that now extend into dendritic spines. It is interesting to note that they have recently been detected at selected synapses (Hu *et al*, 2008; Jaworski *et al*, 2009). These may in turn allow the recruitment of neuronal transport RNPs. Once delivered to the synapse, transcripts are released from the RNPs for translation to occur (Hüttelmaier *et al*, 2005). Hence, the activated synapse is now able to 'capture' plasticity-related molecules from the cell body (Figure 2).

Often multi-subunit complexes are the active molecules at the synapse. Therefore, it is conceivable that (at least) two independent pathways converge to generate such an active complex. One subunit of a key (regulatory) protein is made locally, whereas the other subunit(s) are generated in the cell body and then delivered to the synapse via the sushi conveyor belt. In support of this idea, the active mature CaMKII enzyme consists of two subunits: CaMKIIα and CaMKIIβ (Figure 2). In the cell body, CaMKIIβ is translated and travels to the synapse, whereas CaMKIIα is translated locally only at activated synapses. Thus, the locally synthesized α-subunit might capture the β-subunit, so the functional enzyme is only formed at the activated synapse, generating the synaptic tag. Only when catalytically active, can the kinase initiate downstream pathways yielding the observed remodelling of that particular dendritic spine. In line with this idea is the finding that the functional CaMKII enzyme stays associated with the postsynaptic density within a dendritic spine (Soderling, 2000).

While it may not account for all cases of localized mRNA targeting and translation, and while much remains to be

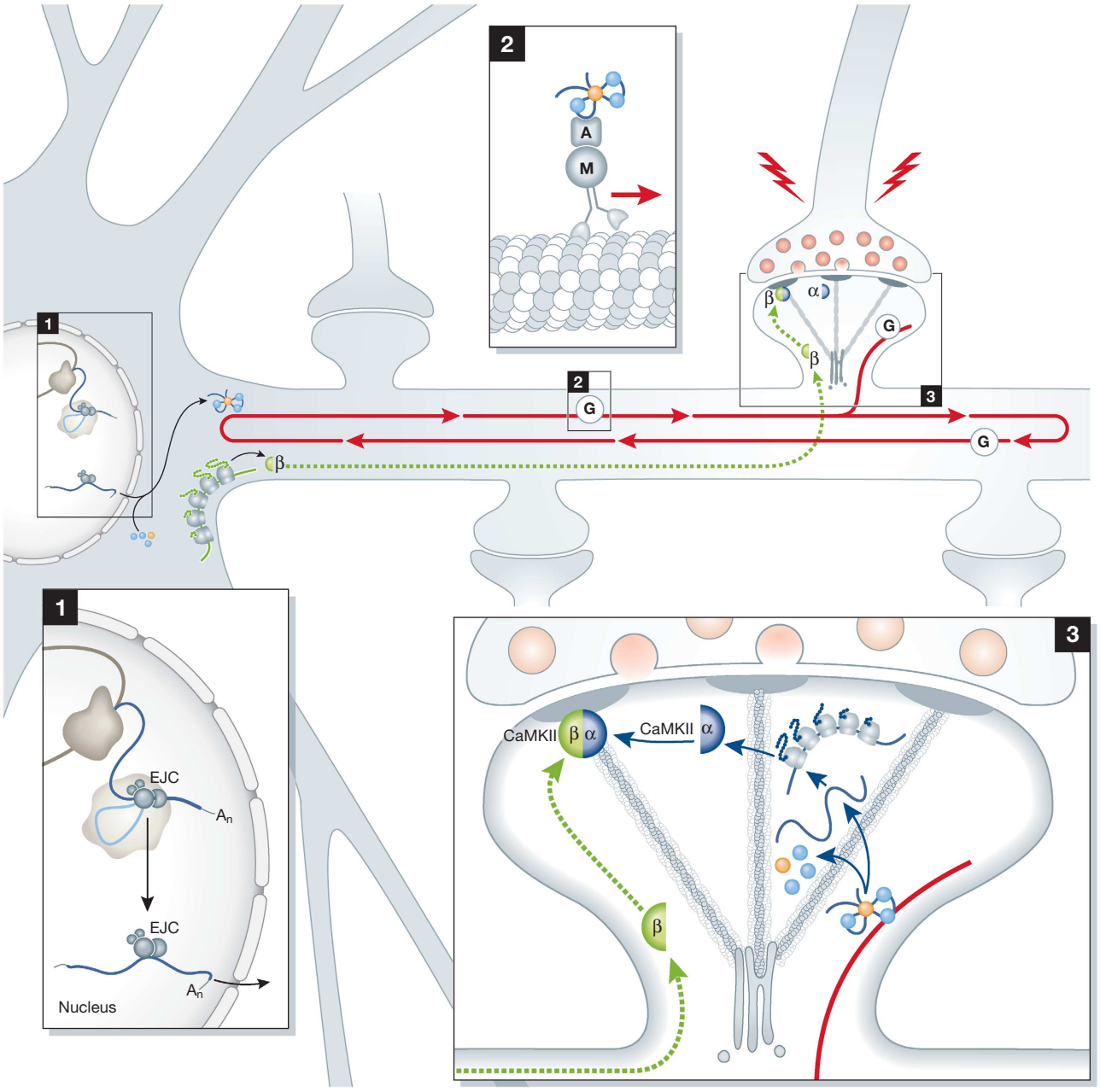

Figure 2 Sushi belt model. RNAs are transcribed in the nucleus of a neuron where they are processed. Next, the EJC is deposited on some intron–exon boundaries before the RNA is exported to the cytoplasm (see inset 1). Once in the cytoplasm—termed cell body of the neuron—the RNP (labelled G for granule) undergoes remodelling and is then transported along the MT cytoskeleton (depicted as a running sushi conveyor belt in red) bidirectionally within dendrites. Such a neuronal RNA granule or RNP typically consists of the transcripts, bound RBPs, adaptor proteins (A) and a molecular motor (M) (see inset 2). In this new model of RNA localization to the synapse termed the '*sushi belt model*', we propose that neuronal RNPs routinely patrol a group of synapses in dendrites like a circling conveyor belt. If a particular synapse becomes activated (represented by inset 3), it may recruit dynamic MTs that now extend into dendritic spines allowing specific delivery of RNPs. This may contribute to a process termed synaptic tagging and capture, initially described by Frey and Morris (1997). Please note that the membrane structure in the neck of the dendritic spine is the spine apparatus, a specialized form of endoplasmic reticulum (inset 3). Ultimately, those synapses upon repetitive activation protocols undergo structural and functional rearrangement. Once delivered to those activated synapses, the RNA is released from the RNP and translated. In many cases, only one subunit of an active protein complex is synthesized locally, whereas the others are made in the cell body and delivered to the synapse by conventional protein transport. One prominent example is the active enzyme CaMKII: the α-subunit (blue semicircle) is made locally where it associates with the β-subunit (green semicircle) that is made and transported from the cell body. Finally, the active enzyme is anchored at the cytoskeleton and/or at the postsynaptic density (grey semiovals underneath the postsynaptic membrane). It should be noted that, while CaMKII is perhaps the best-studied example, other factors may operate similarly in synaptic tagging, and other mechanisms may exist to tag a synapse.

learned about how these processes are coordinated, the sushi belt analogy provides a simple working model. This unites cell body-initiated gene expression, dendritic mRNA localization and local protein synthesis at individual synapses with experience-dependent functional and structural remodelling of synapses during LTM.

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Conflict of interest

The authors declare that they have no conflict of interest.

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