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STUDIES OF mRNA TRANSPORT AND LOCALIZATION

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The cover image illustrates cultured hippocampal neurons transiently expressing exogenous hnRNP CBF-A (green)

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Every problem is an opportunity for creativity

ABSTRACT

During mRNA biogenesis, a subset of newly exported mRNA is assembled into larger RNA granules which are transported to subcellular compartments for localized translation. These mechanisms are critical for asymmetric mRNA and protein distribution and have profound impact on cellular physiology. Yet, they are not fully understood at the molecular level. Transport and localization require cis-acting elements on the RNA that are recognized by cellular transacting factors. The main objective of this thesis has been to elucidate the mechanisms that lead to active mRNA sorting to specialized subcellular compartments for translation.

Cytoplasmic transport and localization of certain mRNAs is mediated by a well characterized cis-acting element termed RNA trafficking sequence (RTS) found in the untranslated regions (UTR). In **paper 1**, we discovered that the heterogeneous nuclear ribonucleoprotein CBF-A binds the RTS in the 3' UTR of the myelin basic protein (MBP) mRNA. CBF-A binding to the RTS occurs both in the nucleus and in the cytoplasm of oligodendrocytes. Since CBF-A gene knockdown impairs MBP mRNA transport to the myelin compartment, we conclude that CBF-A is a novel transacting factor that mediates MBP mRNA transport and localization via the RTS pathway.

In neurons mRNA is present in dendrites and synapses but the mechanisms that mediate transport of specific transcripts are not understood in detail. In **paper 2**, we show that in hippocampal neurons CBF-A functions as transacting factor for transport of Arc, CaMKIIα and BDNF mRNAs to dendrites. This mechanism is RTS-mediated since CBF-A binds to RTS-like sequences in the UTRs of the transcripts and it is dependent on postsynaptic receptor activation. In the nucleus of brain cells CBF-A is excluded from dense chromatin and localizes to nascent pre-mRNPs in perichromatin region. Based on the the fact that CBF-A specifically interact with transcripts that contain RTS sequences, we propose that co-transcriptional RTS-binding by CBF-A may provide de facto a sorting mechanism for transport-competent neuronal mRNAs at an early stage in RNP biogenesis.

In spermatogenic cells expression of haploid mRNA is temporally and spatially regulated, partly by controlling mRNA trafficking. In **paper 3** we report that CBF-A is involved in transport and localization of protamine 2 mRNA during spermatogenesis. CBF-A binds to the conserved RTS in the protamine 2 mRNA 3' UTR and in round spermatids, CBF-A accompanies protamine 2 mRNA to chromatoid body. The larger p42 CBF-A splice variant also appears in protamine 2 mRNA-containing polysomes and interacts with the 5' mRNA cap structure. Since the smaller p37 isoform is excluded from polysomes, we propose that both CBF-A splice variants associate with protamine 2 mRNA and together transit through chromatoid body. In elongating spermatids in response to developmental cue calls when a distinct protamine 2 mRNP emerges in the cytoplasm to engage the translation machinery, the larger p42 isoform remains associated with the transcript via the RTS, presumably to facilitate targeting to the translation machinery.

LIST OF PUBLICATIONS

I. **Chandrasekhar S. Raju**, Christian Göritz, Ylva Nord, Ola Hermanson, Carmen López-Iglesias, Neus Visa, Goncalo Castelo-Branco and Piergiorgio Percipalle. In Cultured Oligodendrocytes the A/B-type hnRNP CBF-A Accompanies MBP mRNA Bound to mRNA Trafficking Sequences
Molecular Biology of the Cell, 2008, Vol. 19, 3008–3019

II. **Chandrasekhar S. Raju**, Nanaho Fukuda, Carmen López-Iglesias, Christian Göritz, Neus Visa and Piergiorgio Percipalle. In neurons, activity-dependent association of dendritically transported mRNA transcripts with the transacting factor CBF-A is mediated by A2RE/RTS elements
Molecular Biology of the Cell ,2011, in press; doi:10.1091/E10-11-0904,

III. Nanaho Fukuda, **Chandrasekhar S. Raju**, Tomoyuki Fukuda and Piergiorgio Percipalle. In mouse testis protamine 2 mRNA transport and localized translation is regulated by the transacting factor CBF-A via the A2RE/RTS pathway during spermatogenesis
Manuscript

Publication not included in this thesis

I. Piergiorgio Percipalle, **Chandrasekhar S. Raju** and Nanaho Fukuda. Actin-associated hnRNP proteins as transacting factors in the control of mRNA transport and localization.
RNA biology, 2009, 6; 171 - 174

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LIST OF ABBREVIATIONS

AMPA	α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrate
ARC	Activity-regulated, cytoskeleton-associated protein
BDNF	Brain derived neurotrophic factor
CaMKIIα	Calcium/calmodulin-dependent protein kinase IIα
CBF-A	CArG box binding factor A
CBs	Chromatoid bodies
DTE	Dentritic target element
EMSA	Electrophoretic mobility shift assay
FISH	Fluorescent in situ hybridization
hnRNPs	Heterogeneous nuclear ribonucleoproteins
LTP	Long-term potentiation
MBP	Myelin basic protein
mRNA	Messenger RNA
NMDA	N-methyl D-aspartate
ORF	Open reading frame
Pre	Premature or precursor
RBPs	RNA-binding proteins
RNA	Ribonucleic acid
RNPs	Ribonucleoproteins
RRM	RNA recognition motif
RLS	RNA localization signal
RTS	RNA trafficking sequence
TAFs	Transacting factors
UTR	Untranslated region

PREFACE

In biology, the question of when and where genes are expressed has been a major focus for at least 60 years. Although initial studies of spatial positioning of transcripts focused on differences in expression levels between tissues, approximately 40 years ago it was first reported that transcripts can also localize asymmetrically within cells (Bodian et al., 1965; Koenig et al., 1965). Interestingly, these early data were initially given little attention. Even after increasing evidence for asymmetrically localized RNAs and ribosome's from independent laboratories in the early 1980s (Colman et al 1982; Jeffery et al 1983; Steward & Levy., 1982) RNA localization continued to be considered something hard to perceive in cell biology. The notion was received with skepticism. However, In the late 1980s and early 1990s it became clear that individual mRNAs can be targeted to distal sites, sometimes even at considerable distances from cell body somatic regions (Verity and Campagnoni., 1988; Shiota et al.,1989; Barbarese et al., 1991). In recent years, the arrival of high throughput approaches has revealed that mRNA localization is much more common than previously thought. A recent study involving large-scale fluorescent in situ hybridization screens indicates that 71% of over 3000 transcripts are expressed in spatially distinct patterns in Drosophila melanogaster embryos (Lecuyer et al., 2007). Similarly, in mammalian neurons hundreds of mRNAs are present in neuronal processes, where they are likely to encode diverse functions (Eberwine et al., 2002; Martin and Zukin., 2006). Therefore, since mRNAs are transported, localized and locally translated in sub cellular domains of many cell types of different eukaryotic species RNA targeting and local protein synthesis provides an important mechanism for regulating gene expression in space and time.

INTRODUCTION

Gene Expression

The chromosomes of an organism contain genes that encode all of the RNA and protein molecules required to construct that organism. Like books in a library, the intention of genes in all living cells is to store information. Gene expression is a series of events through which information encoded in a gene is used to produce a specific protein or a functional RNA (ex. rRNA, tRNA and microRNA). The process of gene expression is irreversible and conserved from prokaryotes (bacteria) to eukaryotes (including multi cellular organisms) and viruses and it is essential for life. The work presented in this thesis comes mainly from mammalian cells, therefore from here onwards I will refer to eukaryote gene expression only.

Eukaryotic gene expression

In multi cellular organisms such as humans, distinct cell types differ dramatically in morphology, structure and function. For instance, when we compare a mammalian neuron with a red blood cell the differences are so extreme that it is difficult to imagine that two cells contain the same genome. Within an individual all cells contain the same genes. Nevertheless, there are hundreds of distinct types of cells in the human body, each expressing a unique set of genes and in turn this unique constellation of expressed genes makes each cell type distinct. Cells may also change the set of genes they express over time and they are constantly adjusting the amount of protein made in response to changing conditions. This is achieved through tight regulation of gene expression at multiple levels (see in fig.1). During expression of protein coding region cells need to integrate and coordinate different layers of control to implement the information in the genome. Thus a cell can control a protein that it makes by (1) transcription control specifying when and how often a given gene sequence is transcribed (2) Processing of the primary transcript altering the synthesized RNA molecule to form a functional mRNA molecule (RNA processing control), (3) selection of which mRNA is exported from the cell to the cytoplasm and determining where in the cytoplasm they are localized (RNA transport and localization), (4) selecting which mRNAs in the cytoplasm are translated by ribosome's (translational control), (5) selectively destabilizing certain mRNA molecules in the cytoplasm (mRNA degradation control), and (6) selectively activating, inactivating, degrading, or compartmentalizing specific protein molecules after they have been made (protein activity control).

During my studies I have focused on post transcriptional aspects of gene expression regulation, mainly on the mechanisms that control cytoplasmic mRNA transport and localization.

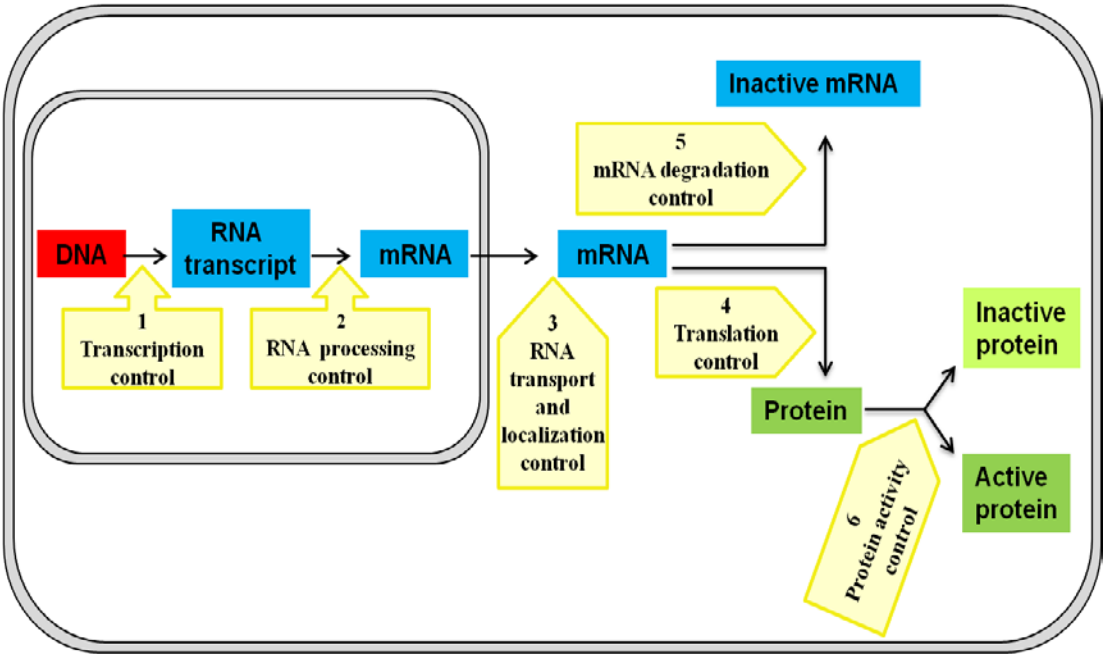

Figure 1. Where can eukaryotic gene expression be controlled? There are multiple steps at which eukaryotic gene expression can be regulated along the pathway from DNA to protein. Regulation of RNA transport and localization (as in point 3) is the focus of this thesis work.

Overview of mRNA biogenesis

Pre-mRNP/mRNP assembly

During mRNA biogenesis, transcription is intimately coupled to co-transcriptional formation of pre-mRNPs and their preparation for nuclear export (Neugebauer et al., 2002; Aguilera et al., 2005; Bentley et al., 2005; Daneholt et al., 2007).

At the gene level, transcription requires alterations of chromatin structure (Groth et al., 2007), which are necessary to lower the nucleosome barrier otherwise imposed on the RNA polymerase II machinery. Changes in chromatin structure are tightly regulated through numerous mechanisms that include histone modification, chromatin remodeling, histone variant incorporation and histone eviction (Li et al., 2007). These events are modulated by co-transcriptional recruitment of co-activators and repressors, they are actin-based and they are necessary for the RNA polymerase II in order to initiate, maintain and terminate mRNA synthesis (Percipalle, 2009). Already at the very

early stages of transcription, nascent mRNA molecules are assembled into pre--mRNPs by heterogeneous nuclear ribonucleoproteins (Dreyfuss et al., 2002). As discussed later on, nowadays it is widely accepted that hnRNP proteins have both a general role in packaging nascent mRNA and also specialized functions. Maturation of nascent pre-mRNPs into mature mRNPs consists of several steps which are also mediated co-transcriptionally by multiple protein factors that become associated with nascent pre-mRNA while being components of specialized molecular machines. These proteins such as splicing factors play an active role in RNP maturation and therefore they are important determinants for mRNA export, localization, translation and stability (Dreyfuss et al 2002; Trcek and Singer, 2010).

pre-mRNA processing

Processing of pre-mRNA to mature mRNA involves the following steps. Normally 5' capping, splicing and 3' end polyadenylation (Alberts, B., 2008). Capping occurs shortly after transcription begins and leads to the formation of 7-methylguanylate (m^7G) to the 5' end of nascent RNA. Cap addition is coupled to transcription, and occurs co-transcriptionally to protect from exonuclease mediated RNA degradation. 5' Capping is also a requirement for nuclear export and importantly it is recognized by the translational machinery. Splicing is the process in which non coding introns are removed and coding exons are joined to one another. It is catalyzed by complexes of proteins and RNA called snRNPs (small nuclear ribonuclearprotein particles) with assemble to form the splicesome. These complexes recognizes special RNA sequences that flank the intron/exon junctions, bind to them and catalyze splicing reactions. Some primary transcripts can be spliced in a few different ways. One such case is alternative splicing which produces range of related proteins referred to as isoforms. To protect or stabilize the 3' end against degradative exonucleases, the mRNA is also cleaved at the 3′ end and several hundred adenosine nucleotides are added. This modification is called polyadenylation and there is evidence that it may enhance translation efficiency.

In addition to these processing events, nucleotides of RNA can be modified either at the ends or internal positions of the RNA molecule, such that they are protected from degradation. Some of these modifications can act as signal to guide the transport of molecule to a particular sub cellular compartment. Some internal modifications, particularly of tRNA and rRNA are necessary for these RNAs to carry out their functions in protein synthesis. Some internal modifications of mRNA change the

sequence of message and so change in amino acid sequence of the protein coded for by the mRNA. After addition of the CAP to the 5 end, the poly-A tail to the 3 end, and splicing of the introns, the processing is complete and the mRNA is exported through nuclear pores to the cytoplasm of the eukaryotic cell where translation will takes place (Alberts. B, 2008).

mRNA export

Normally, once pre-mRNA processing is complete, the translation-ready mRNA is exported from the nucleus to the cytoplasm. The cell therefore requires a mechanism to ensure that only fully processed mRNPs are exported. Traffic of all molecules between the nucleus and cytoplasm essentially occurs via nuclear pore complexes (NPC) that perforate the nuclear envelope. In order to pass through the NPC molecules cargos must associate with different proteins called a transport receptors termed exportins/importins. These transport receptors then act as chaperones that guide cargos through the NPC. Recognition by the transport receptor takes place via a specific sequence of amino acids in the cargo protein called Nuclear localization signals (NLSs) and nuclear export signals(NESs). Besides cargo and transport receptors, another ingredient necessary for Nucleocytoplasmic transport is the signaling protein Ran, which hydrolyzes GTP (Cole et al., 1998). Ran is responsible for regulating the interaction of transport receptor and cargo and RanGDP/RanGTP concentration gradients across the nuclear envelope drive nuclear import and export. The mechanism by which the NPC selectively allows the transit of import or export complexes, while restricting the others is poorly understood. However, nuclear pore complex proteins called nucleoporins (Nups) thought to have been implicated in this process. Normally, once pre-mRNA processing is complete mRNA is exported from the nucleus to the cytoplasm for translation. mRNA export is a three step process involving the generation of a cargo-carrier complex in the nucleus followed by translocation across the nuclear pore complex and finally release of the cargo in the cytoplasm. The TAP/ NXF1:p15 heterodimer is a key player in mRNA export. Overexpression of TAP increases the export of transcripts . As both TAP and p15 show low affinity for RNA, they associate with adaptor proteins, presumably RBPs (RNA Binding Proteins) to mediate the interaction (Katahira et al., 1999). The Aly/REF protein which directly interacts with TAP and recruits TAP to mRNA, although the precise mechanistic details of mRNA export remain unclear (Taniguch et al., 2008).

Heterogeneous nuclear ribonucleoproteins in mRNA biogenesis

As mentioned above, immediately upon transcription pre-mRNA molecules associate with proteins to form ribonucleoprotein (RNP) complexes, usually referred to as pre-mRNP or hnRNP (heterogeneous nuclear RNP) particles. These proteins are designated hnRNP proteins and have been extensively characterized (Dreyfuss et al., 1993; Dreyfuss et al., 2002, Daneholt, 1997). In human, for example, the family of hnRNP proteins includes about twenty major components and a large number of minor ones (Krecic and Swanson, 1999). In vitro the various hnRNP proteins bind to RNA in a general manner but show sequence preferences. Binding to heterogeneous nuclear RNA is non-random, and each transcript seems to carry a specific subset of proteins. It is likely that the hnRNP proteins are involved in RNA packaging. Furthermore hnRNP proteins are likely to determine the fate of RNA. Several hnRNP proteins, including hnRNP A1, F, H, and I, are known to regulate splicing and 3´-end processing (Krecic and Swanson, 1999). The composition of hnRNP proteins in RNPs highly dynamic and influence its downstream steps. The hnRNP proteins that are bound on intronic sequences are removed after splicing, leaving behind only the hnRNP proteins that are bound to exonic sequences. Some hnRNPs contain nuclear retention signals and are removed from the mRNP prior to nuclear export(Nakielny and Dreyfuss, 1996), where as others remain associate with mRNP all the way from gene to polysomes to be shuttled between nucleus and cytoplasm. Thus, hnRNP composition in RNPs is highly dynamic and rearranges throughout the mRNA biogenesis pathway as can be seen in fig 2.

The most abundant hnRNP proteins belong to the A/B type and exhibit a well defined modular structure. The N-terminal domain is highly conserved and consists of two tandemly repeated, 80–90 amino acid long, RNA-binding domains (RBD). The C-terminal part of the protein is considerably more divergent, is glycine-rich, and is called the auxiliary domain. Therefore, the A/B-type proteins are often referred to as 2xRBD-Gly proteins. These proteins constitute a continuously expanding family of proteins with multiple post-transcriptional isoforms as well as extensive post-translational modifications. They have a general function in RNA processing and transport as well as specialized functions. hnRNP A1 and A2/B1 proteins participate in constitutive (Sierakowska et al., 1986) and alternative splicing (Chabot et al., 1997) where they seem to antagonize the SR splicing factor ASF/SF2 in the selection

of the 5 splice site (Mayeda and Krainer, 1992). Certain A/B type hnRNP proteins are also known to shuttle between nucleus and cytoplasm (Pinol-Roma and Dreyfuss, 1992). Because of This property it was proposed that hnRNP proteins could also mediate RNA transport; this view was further substantiated by evidence that an hnRNP A1-like protein travels with the RNA through the nuclear pore (Visa et al., 1996). Subsequently, nuclear export signals were identified in both hnRNP A1 (Michael et al., 1995) and hnRNP K (Michael et al., 1997), but it is not known whether these proteins bind directly to the nuclear pore complex or via a soluble receptor (Nakielny and Dreyfuss, 1999).

The observation that certain hnRNP proteins accompany the mRNA into cytoplasm indicates that they may be involved in the regulation of mRNA translation. It is known that general RNA-binding proteins, such as hnRNP A1, render translation cap-dependent and prevent internal initiations along the message by coating the mRNA (Svitkin et al., 1996). The hnRNP A2 protein, bound to a specific sequence in the 3´ end of myelin basic protein mRNA, enhances cap-dependent translation (Kwon et al., 1999). There is also evidence that mRNA stability, mRNA targeting to specific cytoplasmic locations requires hnRNP proteins. Both hnRNP C (Zaidi and Malter, 1995) and hnRNP D (Kiledjian et al., 1997; Loflin et al., 1999) bind to AU-rich elements which are known to regulate mRNA turnover. In Drosophila melanogaster the Squid protein (hrp40), an hnRNP A1-like protein (Kelley et al., 1993; Matunis et al., 1994), governs the localization specific mRNAs, such as grk mRNA, during oogenesis (Kelley et al., 1993; Matunis et al., 1994) and it is essential for localization of transcripts the apical cytoplasm in Drosophila blastoderm embryos (Lall et al., 1999). In mammals, the hnRNP A2 protein has been implicated in directed transport of myelin basic protein (MBP) mRNA to the distal ends of the cytoplasmic processes of mammalian oligodendrocytes (Hoek et al., 1998).

We recently discovered that the CArG box binding factor A (CBF-A) which was originally identified as transcriptional regulator (Kamada and Miwa, 1992; Bemark et al., 1998; Mikheev et al., 2000) is also a genuine hnRNP protein of the A/B type that displays considerable homology when compared to hnRNP A2. CBF-A, which is also denoted hnRNP A/B, is abundantly present in the 40S pre-mRNP/mRNP fraction isolated from rat liver extract and it is therefore considered as a core hnRNP protein. We discovered that CBF-A binds poly A mRNA in nucleus and cytoplasm and rapidly

shuttles between the two cellular compartments. Interstingly, within the RNP particle CBF-A was found to directly interact with actin in nucleus and cytoplasm, suggesting that it is likely to accompany mRNA from gene to polysomes (Percipalle et al., 2002). CBF-A is also known to come into at least two well characterized isoforms namely p37 and p42 that are generated by alternative splicing of the same transcript (Dean et al., 2005). However at this stage it is unclear whether the two variants display different functions.

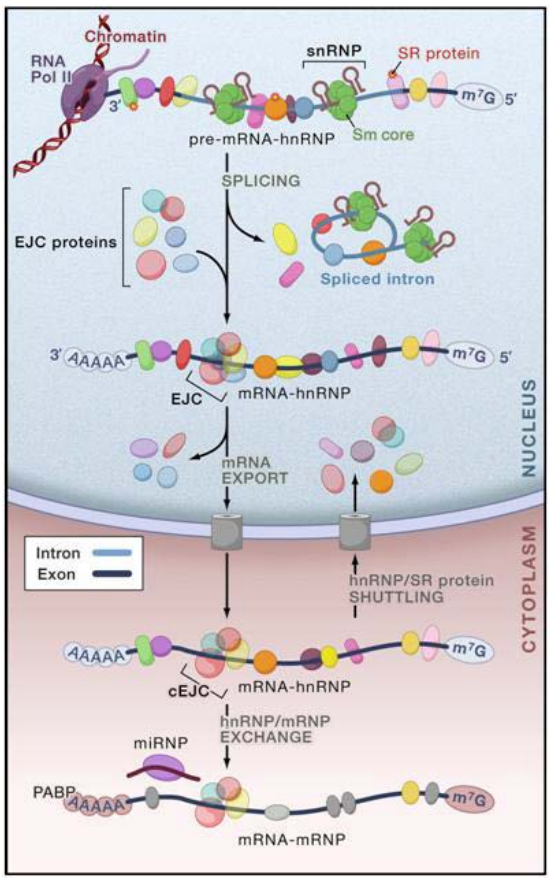

Figure 2. Posttranscriptional gene regulation and hnRNP proteins along the pathway of mRNA biogenesis. The protein composition of pre-mRNA and mRNA is highly dynamic and changes throughout mRNA biogenesis. RNA pol II, RNA polymerase II; hnRNP, heterogeneous nuclear ribonucleoprotein; SR, serine-arginine-rich protein; m7G, 7-methyl guanosine; snRNP, small nuclear ribonucleoprotein; mRNP, mRNA–protein complex; EJC, exon junction complex; PABP, poly(A)-binding protein. (Adapted from Cooper, T.A., and Dreyfuss, G. 2009)

Cytoplasmic mRNA transport and Localization

After export from nucleus to cytoplasm, certain eukaryotic messenger RNA (mRNA) molecules do not undergo translation immediately, instead they are directed to specific sub cellular domains for translation. RNA Transport and localization is a widespread phenomenon that has been observed in many cell types of different species as shown in Fig 3. To date, the best studied examples of mRNA localization explore transcripts whose protein products play specialized roles within well defined sub cellular

compartments. In budding yeast, many RNAs translocate from the mother cell into the budding daughter cell and concentrate at the bud tip (Shepard et al., 2003). One of the best studied examples of yeast is mRNA encoding the transcriptional repressor ASH1 (Long et al., 1997; Takizawa et al., 1997). ASH1 mRNA is transported to the bud tip of dividing cell such that it is only delivered to nucleus of daughter cell, thereby ensuring daughter and mother cells have distinct mating types (Paquin and Chartrand., 2008).
In Drosophila, the localization of mRNAs, such as bicoid, oskar, and nanos to anterior and posterior poles of the oocyte, helps to establish morphogen gradients that underlie the proper spatial patterning of the developing embryo (Johnstone and Lasko., 2001). Similar processes occur in Xenopus, where several mRNAs are localized to the different poles of oocytes. mRNA encoding the T-box transcription factor VegT localizes to the vegetal pole of frog oocytes during oogenesis and induces endodermal and mesodermal cell fates in the embryo (Melton.,1987; King et al., 2005).

In mammals, several mRNAs are localized to specific sub cellular regions or domains. Some of the best characterized examples are mRNAs encoding β-actin and myelin basic protein (MBP). In migrating fibroblasts, β-actin mRNA localizes to the leading edge (lamellipodia) of the cells (Lawrence and Singer, 1986), where its local translation is necessary for cytoskeletal-mediated motility (Condeelis and Singer, 2005). Similarly, β-actin mRNA is also localized to neuronal growth cones and dendritic filopodia, filopodial synapses (Bassell et al., 1998; Eom et al., 2003). In oligodendrocytes, MBP mRNA is targeted to the distal processes where myelination occurs (Ainger et al., 1993, Smith., 2004). In neurons, CamKIIα mRNA is localized to dendrites (Mori et al., 2000; Miller et al., 2002) where its local translation is required for neuronal plasticity. During brain development, local axonal translation of mRNAs and new protein synthesis may function in growth cone mediated axon guidance (Lin and Holt, 2007). Similarly In the mature brain, the regulated translation of dendritically localized mRNAs allows each of the thousands of synapses produced by a given neuron to independently regulate its structure and function during synaptic plasticity, thereby greatly increasing the computational capacity of the brain (for review, see Martin and Zukin, 2006; Martin, and Ephrussi, 2009).

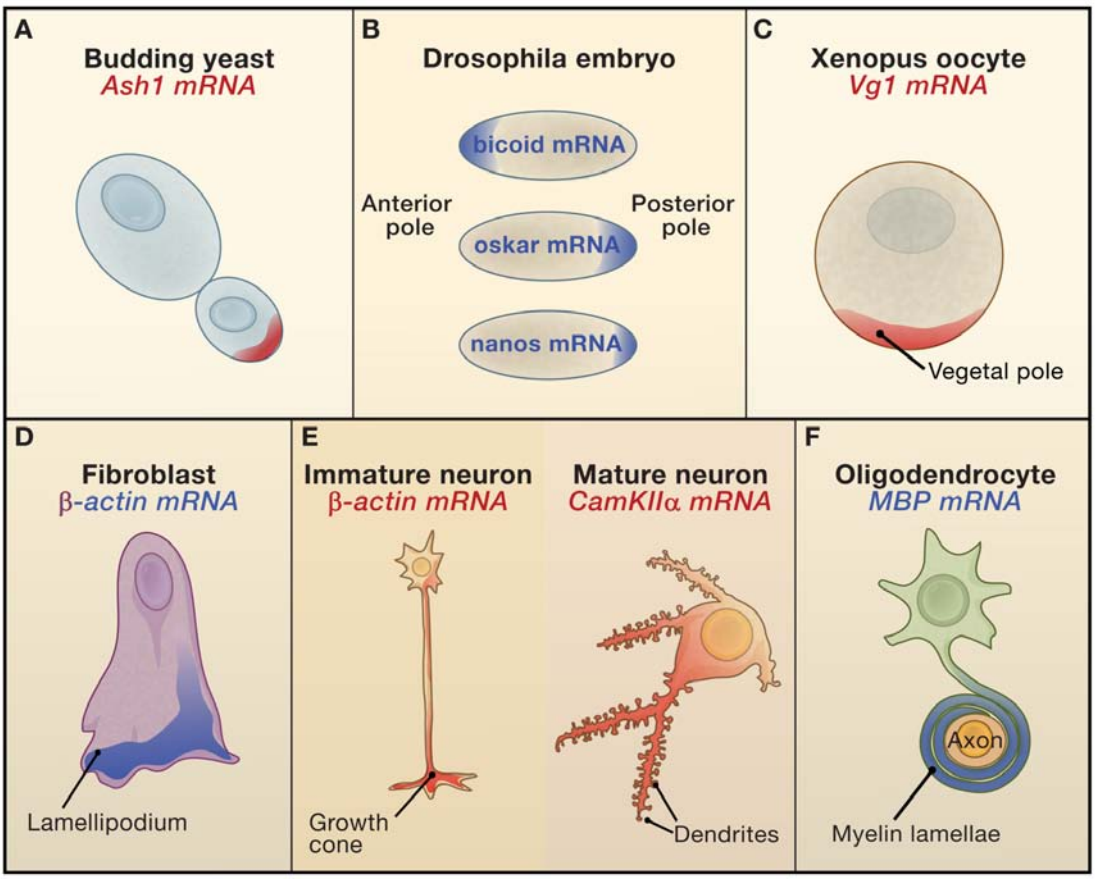

Figure 3: Examples of localized mRNAs in many cell types of different species. A) In budding yeast, the ASH1 mRNA localizes to the bud tip. B) In Drosophila embryos, bicoid mRNA localizes to the anterior pole, oskar and nanos mRNAs to the posterior pole. C) In Xenopus oocytes ,Vg 1 mRNA localizes to the vegetal pole. D) In chicken and mammalian fibroblasts, β-actin mRNA localizes to lamellipodia. E) In developing, immature mammalian neurons, β-actin mRNA is present in distal growth cones; in mature neurons, CamKIIα mRNA is present in distal dendrites. F) In mammalian oligodendrocytes, myelin basic protein (MBP) mRNA localizes to myelinating processes, that ensheath neuronal axons (Adapted from Martin, K.C. and Ephrussi, A. 2009).

Biological significance of RNA sorting

RNA localization not only targets the protein to the appropriate region of the cell, but also prevents its expression elsewhere. For instance, the mislocalization of oskar or nanos mRNAs in the Drosophila egg induces the development of a second abdomen in the place of the head and thorax (Ephrussi et al., 1991; Gavis et al., 1992). For other localized mRNAs, it might not be possible to localize the encoded proteins to the correct compartments, because they bind to other factors wherever they are made. This is the case for Tau and MAP2, which will bind to any microtubules, and must therefore be localized as mRNAs to axons or dendrites, respectively (Aronov et al., 2001).

There are several reasons why targeting mRNAs could be advantageous over targeting the protein product directly (for reviews, see St Johnston et al., 2005; Martin and Ephrussi, 2009).

1. RNA localization provides a mechanism for gene expression to be spatially restricted within the cytoplasm.
2. Spatially restricted gene expression can be achieved with high temporal resolution in response to local stimuli or extrinsic cues. Local stimuli can regulate translation on-site, without sending signal to the nucleus to initiate transcription, followed by mRNA export, cytoplasmic translation, and subsequent targeting of the protein to the site of stimulation.
3. Increased cost effectiveness because of the production of multiple protein copies from single localized mRNA molecules.
4. RNA targeting and local translation prevents proteins from acting ectopically during their translocation.
5. Facilitating the assembly of macromolecular protein complexes by producing a high local concentration of mRNA molecules in sub cellular domains.

The importance of cis-acting elements for mRNA transport and localization

Eukaryotic mRNAs share common features that include exons and introns, 5' and 3'-untranslated regions (UTRs). Most mRNA regulatory elements are located within the 5' and 3'UTRs, where they act as platforms for the assembly of protein complexes to the mRNA, thereby generating ribonucleoparticles (mRNPs). The 5'UTR is primarily involved in controlling mRNA translation (Pickering et al., 2004), whereas 3'UTR regulates multiple aspects of mRNA metabolism, including nuclear export, cytoplasmic localization, translational efficiency and mRNA stability (Moore et al., 2005). The targeting of mRNA to specific subcellular compartments is mostly determined by cis acting elements that are located in the 3' UTR, although in some cases they are present in the 5′UTR or in the coding sequence . Cis-acting elements are likely to express as a segments or motifs within RNA that contain codes to specify functionality (e.g., cellular targeting) and often named transport and localization elements or zipcodes. The first evidence indicating that cis-acting RNA elements are required for transport and localization involved genetic deletion analysis coupled with microinjection studies. In these studies elements of the localized mRNAs were fused to hybrid genes in order to identify sequences that were required for localization. These and many other subsequent reports revealed that localization elements are most often found in the 3′UTR and can range in length from five or six to several hundred nucleotides. Different studies aimed at identifying cis acting elements led to following principles

that these are often exist as multiple copies of the same element or as a combination of different elements (Jambhekar and Derisi, 2007). Distinct localization elements mediate distinct steps in localization and localization elements can form secondary structures, usually stem loops that are critical for localization. Cis-acting elements can promote localization in three different ways: 1) by active and directed transport of the transcript to a sub cellular site (the most common mechanism described to date); 2) by mediating the local stabilization and regulated degradation of mRNAs and 3) by locally trapping an mRNA that diffuses throughout the cytoplasm (for reviews, see St Johnston et al., 2005; Martin and Ephrussi, 2009).

In Drosophila, systematic approaches led to identification of cis acting elements that mediate bicoid mRNA localization to the anterior pole. First it was reported that 625 base pairs of the 3′UTR are responsible for anterior localization of bicoid mRNA in Drosophila melanogaster embryos (Macdonald and Struhl., 1988). By expressing transgenes containing smaller deletions several elements (called bicoid localization elements, or BLEs) were identified within this 625 bp region that were necessary for localization (Macdonald et al., 1993). One of these elements BLE1, consisting of 50 nucleotides formed a stem loop structure that was specifically required for bicoid mRNA transport from the nurse cells into the oocyte (Ferrandon et al., 1997). Furthermore it was shown that BLE1 directs the early steps of bicoid mRNA localization. Additional stem loop structures were required for later steps in localization and still an additional stem loop was required for RNA anchoring at the anterior pole (Ferrandon et al., 1997; Macdonald and Kerr, 1997). Mutations that disrupt the primary sequence of the localization element BLEs, but not the stem-loop structure formation were shown to permit mRNA localization (Ferrandon et al., 1997), demonstrating the secondary structure plays critical role in mRNA localization. Finally, it was shown that bicoid mRNA dimerizes in vitro through interactions between specific hairpin loop structures and this dimerization was shown to be essential for binding of the RNA binding protein Staufen in embryo injection assays (Ferrandon et al., 1997). Staufen, in turn is required for bicoid mRNA localization to anterior pole during the late stages of oogenesis (St Johnston et al., 1991; Weil et al., 2006), suggesting that dimerization is an important step in bicoid localization.

In Xenopus oocytes, microinjection of transcripts containing elements of the Vg1 RNA revealed that 340 nucleotides of the 3′UTR were required to localize the mRNA to the

vegetal pole (Mowry and Melton, 1992). Comparison studies of this region between two frog species revealed two 5–6 nucleotide-long sequences, called VM1 and E2 elements or motifs (Lewis et al., 2004). The Vg1 3′UTR contains multiple, clustered copies of these localization elements that act synergistically to localize Vg1 mRNA (Deshler et al., 1997; Lewis et al., 2004). In Yeast the cis-acting sequences that mediate ASH1 mRNA targeting to the bud tip provide another example of repetitive and synergistic clustering of localization elements. ASH1 mRNA contains four localization elements, three of which (E1, E2A, E2B) are in the coding sequence of ASH1, whereas the fourth (E3) overlaps with the coding sequence and 3′UTR (Chartrand et al., 1999; Gonzalez et al., 1999). These elements were all predicted to form stem-loop structures and mutations that disrupt secondary structure formation are not localization competent. Each element on its own is capable of localizing a reporter RNA, although the presence of four elements increases the efficiency of localization (Chartrand et al., 2002).

In chicken fibroblasts and myoblasts, β–actin mRNA was found to localize to the leading edge of the cells (Lawrence and Singer, 1986). Different approaches to define cis-acting sequences in β–actin mRNA, revealed that a 54 nucleotide-long sequence in the 3′UTR is essential and sufficient for mRNA localization (Kislauskis et al., 1994). This element was termed the "zipcode" because it contains the cytoplasmic delivery address for transport. Comparison studies among β–actin transcripts from many other species revealed, conserved hexanucleotide sequence ACACCC (Kislauskis et al., 1994). Chicken β–actin mRNA contains tandem repeats of this hexanucleotide motif and mutations in this region inhibits localization (Ross et al., 1997). Secondary structure analysis predicted that the β-actin zipcode forms a stem-loop structure .

Synergies between cis-acting elements and transacting factors for mRNA transport and localization.

Transport and localization depends not only on the mRNA cis acting element, but also on cellular factors that specifically interact with these sequences to form the critical RNP complex. Trans-acting factors (TAFs) by definition are proteins that bind to cis-acting elements to facilitate mRNA transport and localization to their subcellular destinations. Identification of transacting factors that are involved in mRNA trafficking has emerged primarily from two kinds of studies, genetic screens for genes involved in mRNA localization and affinity isolation of proteins that bind the identified localization

elements. In genetic screens, Staufen was first identified because of its role in pattern formation, reflecting its function in localizing oskar and bicoid mRNAs in Drosophila oocytes (St Johnston et al., 1991). Staufen is one of the best studied transacting factor and it is known that it directly binds a variety of mRNAs. It binds to stem-loop structures within the bicoid mRNA 3'UTR and is required in order to anchor bicoid (bcd) mRNA at the anterior pole (Ferrandon et al., 1994). Similarly, oskar (osk) mRNA is localized at the posterior pole, most likely through the interaction of Staufen with the oskar 3′UTR (Jenny et al., 2006), a mechanism that occurs at two distinct stages of development. Its mammalian homolog is also involved in the targeting of mRNAs, such as CamKIIα mRNA, to neuronal dendrites (Kiebler et al., 1999; Tang et al., 2001) supporting the idea that mRNA localization is evolutionarily conserved (Roegiers and Jan, 2000). Staufen has 5 distinct double-stranded RNA binding (dsRNA) domains (four in mammalian Staufen) each of which binds dsRNA non specifically (St Johnston et al., 1992) indicating that additional proteins may be recruited to the RNP to achieve specificity (Ferrandon et al., 1994; St Johnston et al., 1992). Domain 2 is essential for microtubule-dependent localization of oskar mRNA (Kim-Ha et al.,1991; Ephrussi et al.,1991) and domain 5 is required for the interaction with Miranda protein that allows actin-dependent localisation of prospero mRNA (Matsuzaki et al., 1998; Schuldt et al.,1998).

Affinity isolation methods led to the identification of zipcode-binding protein (ZBP1) that binds to the β-actin mRNA zipcode in chicken fibroblasts (Ross et al., 1997). ZBP1 features two RNA recognition motifs (RRM) and four hnRNP K homology (KH) RNA binding domains. For each of these domains, different functions have been identified. Specifically, the KH domains (3 and 4) mediate binding to the zipcode, formation of an RNP and association with actin microfilaments (Farina et al.,2003), whereas the RRM domains are required for the localization of the β-actin RNP.

ZBP1 Homologs have been identified in Xenopus, Drosophila, mouse and human and implicated in mRNA localization in each case. The ZBP1 homolog Vera binds to localization elements in Vg1 3′UTR and is required for localization to the vegetal pole in Xenopus oocytes (Deshler et al., 1997). ZBP1 is also present in mammalian neurons, where it binds β-actin mRNA (Zhang et al., 2001). In developing neurons, ZBP1 localizes to growth cones, where stimulus induced local translation of β-actin is required for growth cone navigation (Lin and Holt, 2007). In mature neurons, ZBP1

undergoes activity-dependent trafficking and dynamic localization in dendrites and spines (Tiruchinapalli et al., 2003). Phosphorylation by src kinase, results in reduced binding of ZBP1 to RNA and to increased translation of β-actin in neuroblastoma cells (Huttelmaier et al., 2005). Altogether, these findings suggest that ZBP1 functions both in mRNA localization and translational repression. ZBP2, another zipcode binding protein was recently identified by affinity purification (through binding to the zipcode) and is a predominantly nuclear protein that also affects β-actin localization in the cytoplasm (Gu et al., 2002). ZBP2 is a homologue of human nuclear RNA splicing factor KSRP. Like ZBP1, ZBP2 orthologs identified in other species have also been shown to be involved in mRNA localization.

In rat, the homolog of ZBP2 is MARTA1 (MAP2-RNA Trans-Acting protein 1), which binds the 3′UTR of MAP2, a dendritically localized mRNA in neurons (Rehbein et al., 2000). In Xenopus, the ZBP2 homologue VgRBP71 also binds the Vg1 mRNA, which is localizes to the vegetal pole of the egg (Kroll et al., 2002). Recent work on chicken ZBP2 indicated that ZBP2 co-transcriptionally binds the nascent β-actin zipcode, and facilitates the binding of ZBP1 to the zipcode (Pan et al., 2007). Therefore, these results suggest that the role for ZBP2 in the nucleus provides support to the working model that the co transcriptional recruitment of proteins with the RNA is required for ultimate localization in the cytoplasm. It also provides an example of how interactions between RNA binding proteins and RNA serve to recruit and stabilize additional proteins to form a large RNP. One consequence of this cooperative binding is that it becomes critical to analyze the function of individual RNA binding proteins in cells that are null for the endogenous protein (Pan et al., 2007).

In mammalian oligodendrocytes, the identification of RNA trafficking sequence (RTS) in 3'UTR of MBP (myelin basic protein) mRNA that mediated transport and localization to myelinating processes allowed the use of synthetic oligonucleotides containing this sequence to affinity purify RTS binding proteins from rat brain (Hoek et al., 1998). Among these proteins hnRNP A2 was found to be involved in MBP mRNA transport and localization and also in the different mRNAs of dendrites of (Shan et al., 2003; Gao et al., 2008). hnRNP I shares 87% amino acid sequence identity with the Xenopus VgRBP60 protein, which binds the VM1 element of the Vg1 mRNA 3'UTR and colocalizes with this messenger (Cote et al , 1999). The Drosophila Squid protein is a homologue of hnRNP A1 and it is required to localize gurken mRNA to the anterior

dorsal cortex of the oocyte through binding to two distinct regions of the 3'UTR (Norvell et al., 1999).

Nuclear events for cytoplasmic localization

In Drosophila, nuclear shuttling proteins Y14/Tsunagi and Mago nashi are required for oskar mRNA localization, and they co-localize with oskar mRNA at the posterior pole of the oocyte (Hachet et al, 2001; Mohr et al, 2001). Their human homologues, Y14/RBM8 and Magoh, are core components of the exon–exon junction complex (EJC). The EJC is deposited on mRNAs in a splicing-dependent manner, 20–24 nucleotides upstream of exon–exon junctions, independently of the RNA sequence. That indicated a possible role of splicing in oskar mRNA localization, challenging the established notion that the oskar 3' untranslated region (3'UTR) is sufficient for this process. However, later research demonstrated that the accurate localization of oskar mRNA to the oocyte posterior pole was shown to depend on the presence of the first intron and splicing at the first exon–exon junction of oskar RNA is necessary (Hachet and Ephrussi, 2004), in addition to the 3′UTR. The fact that both splicing and the EJC components Y14 and Mago nashi are essential for oskar mRNA localization, provides striking example that RNA splicing and cytoplasmic localization are mechanistically coupled by the splicing dependent deposition of the EJC.

Cellular infrastructure for RNA transport

For localization, mRNA must be targeted to appropriate cytoplasmic compartments. This is an especially daunting task given the long distances that may separate nuclear envelope and cellular periphery. Therefore there is a requirement for an active mRNA transport mechanism which also provide directionality. It is now widely accepted that mRNA transport requires molecular motors to be delivered to their final destination. Whether messenger ribonucleoproteins (RNP)s on the move contain single or multiple RNAs and how they are linked to motors is still a matter of concern, but recent developments in live fluorescence imaging, RNP purification methods and genetic studies verified that active, motor protein-dependent transport occurs both along actin and microtubule filaments. Several recent publications indicated that these cytoskeletal networks provide highways for trafficking of mRNPs within the cytoplasm as shown see in fig.4. Molecular motor proteins such as kinesin, dynein, and myosin facilitate RNA trafficking along microtubule and actin filaments. Thus, active transport of

mRNAs along cytoskeletal filaments has been implicated as the major localization mechanism in most cells (Carson et al., 1997; Kanai et al., 2004; Singer et al., 2008). In this context, I will list below few model examples that have been described in details.

As I mentioned above briefly, In budding yeast, many RNAs are targeted from the mother cell into the budding daughter cell. For instance, the localization of ASH1 mRNA to the budding yeast provides a model example of myosin mediated mRNA transport along actin filaments. She3p, one of the RNA binding proteins required for the localization of ASH1 mRNA, acts as an adaptor that links the ASH1 mRNA to the motor protein Myo4p (also called She1p). Myo4p belongs to class V myosins and has been shown to direct transport of substrates along actin microfilaments in living yeast (Reck-Peterson et al. 2001). Yeast mutants that prevent bundling of actin filaments have been shown to result in mislocalization of ASH1 mRNA (Long et al., 1997; Takizawa et al., 1997). In addition to a role for actin in the targeting of ASH1 mRNA, actin plays a important role in anchoring ASH1 mRNA at the tip . Disruption of cortical actin at the bud tip alters ASH1 mRNA localization (Beach et al., 1999). The actin cytoskeleton is also shown to be important for targeting of a number of other localized mRNAs, including, bicoid mRNAs in Drosophila oocytes and embryos (Weil et al., 2008) and Vg1 mRNA in Xenopus oocytes (Yisraeli et al., 1990). Recently it was reported that actin polymerization is required for Arc/Arg3.1 mRNA targeting to activated synaptic sites on dendrites (Huang et al., 2007).

In fibroblasts, actin microfilaments are used to localize RNP particles that contain β-actin transcripts and ZBP1 to the leading edge (Farina et al., 2003), On the other hand, in neurons, ZBP1 and its β-actin mRNA target seem to move predominantly along microtubules (Bassell&Kelic 2004). Thus ZBP1 may act as an adaptor between mRNA and either microfilament- or microtubule based molecular motors. It is possible that in neurons, long-range RNA transport is mediated by microtubules, whereas localization in the destination microdomain is supported by actin filaments (Muslimov et al. 2002).

In oligodendrocytes and neurons, microtubules and corresponding motor proteins have been demonstrated to play a critical role in transporting RNPs or RNA granules to distal sites. As one example, in living neurons it was first reported that recruitment of staufen green fluorescent protein to large RNA containing granules and subsequent dendritic transport is dependent on microtubules (Kiebler et al., 1999; Tang et al., 2001). Microtubule anterograde motor KIF5 is implicated in transporting many

dendritically localized transcripts and further it was showed that distally directed movement was enhanced by the over expression of KIF5 and reduced by its functional blockage. In addition, kinesin (KIF5) associated complexes from mammalian brain were shown to contain several of the above mentioned TAFs (Kanai et al. 2004) Neuronal CPEB granules contain both kinesin and dynein motors, and their bidirectional movement in dendrites is microtubule dependent (Huang et al. 2003). In the axon-like processes of P19 embryonic carcinoma cells, knock-down of another kinesin family member, KIF3A, impairs the sorting of tau mRNA (Aronov et al. 2002). In oligodendrocytes, inhibition of kinesin disrupts targeting of MBP mRNAs (Carson et al. 1997). In testis, kinesin KIF17b associates with TB-RBP, suggesting that a microtubule dependent RNA transport system operates in mammalian male germ cells (Chennathukuzhi et al. 2003).

Inhibition of kinesins have been shown to inhibit FMRP (Fragile X mental retardation protein) transport into dendrites, and have further indicated that FMRP interacts with at least two distinct kinesin isoforms (Dictenberg et al., 2008). The finding that FMRP can use two kinesin motors indicates that molecular motors may play redundant roles in mRNA transport. Consistent with such redundancy, a recent study from Mowry and colleagues has shown that multiple kinesins coordinate the transport of mRNAs in Xenopus oocytes (Messitt et al., 2008).

Evidence is thus accumulating that long-range mRNA transport in various mammalian cell types is mediated by microtubule based kinesin and dynein type molecular motors. In many cases, the mRNA is anchored/localized at its final destination in an actin dependent manner (for review, see Kindler et al., 2005)

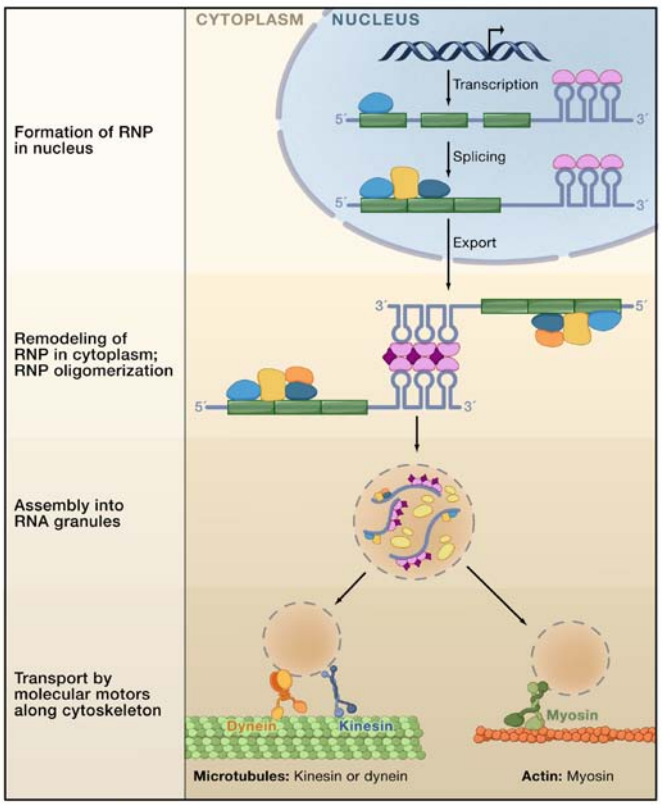

Figure 4. Cellular infrastructure for mRNA transport and localization. Following RNP export into the cytoplasm, the RNPs are remodeled and assembled into RNA granules. These RNA granules are then transported along cytoskeletal networks including microtubules and actin filaments, using molecular motors such as kinesin, dynein and myosin (Adapted from Martin, K.C. and Ephrussi, A. 2009).

Spatial and temporal regulation of mRNA transcripts

Lessons from Brain

In brain, oligodendrocytes and neurons are two post mitotic cells that have been extensively used to study mRNA transport and local protein synthesis. Both cell types contain several processes or dendrites/dendritic spines in a given cell and many different mRNAs are targeted to these subcellular compartments. In both of these cell types certain genetic information is targeted from the nucleus to processes or dendrites in the form of RNA granules or RNP granules. In oligodendrocytes these granules contain RNAs coding for proteins required for myelination which insulates axons in the central nervous system. In neurons these granules contain RNAs coding for proteins required for axonal growth and Synaptic plasticity. Although these cells differ significantly in the molecular and cellular properties and in the morphology and function between processes and dendrites/dendritic spines the process of translocation of RNA molecules from the nucleus to periphery appears to be similar.

RNA trafficking in myelinating oligodendrocytes

The first experimental evidence indicating one of the major component of myelin was synthesized near its site of assembly in the myelin sheath rather than in the cell body came from pulse-labelling studies and further it was revealed that newly synthesized MBP reached the myelin compartment within minutes of translation, whereas incorporation of other myelin proteins such as proteolipid was delayed (Benjamins & Morell, 1978). Subsequently, it was determined that isolated myelin fractions are highly enriched for mRNAs coding MBPs but not in mRNA for PLP (Colman et al., 1982). Localization of MBP mRNA near sites of myelin assembly was later confirmed by in situ hybridization both in vivo and in cultured cells (Kristensson et al., 1986; Verity and Campagnoni, 1988). Co-culture studies indicated that localization of MBP mRNA to the myelin compartment was facilitated by neurons and inhibited by astrocytes (Landry et al., 1994) through a PDGF based mechanism (AmurUmarjee et al 1997). Besides MBP mRNA, several other mRNAs including, myelin-associated oligodendrocytic basic protein (MOPB) mRNA (Holz et al., 1996), tau mRNA (LoPresti et al., 1995), carbonic anhydrase II (CAII) mRNA (Ghandour & Skoff, 1991; Tansey et al., 1996) and amyloid precursor protein (APP) mRNA (Garcia-Ladona et al.,1997) are also localized to the myelin compartment or the distal processes of oligodendrocytes.

RNA granule hypothesis

The early work on RNA localization in myelinating cells mainly came from in situ hybridization studies. This approach only yielded information regarding the steady state sub cellular distribution of RNA, but not much known about the dynamic aspects of RNA trafficking, in living cells. High resolution fluorescence *in situ* hybridization (FISH) revealed that endogenous MBP mRNA appears as discrete particulate structures all along the oligodendrocyte processes (Verity and Campagnoni, 1988; Ainger et al., 1993). Such organization of MBP mRNA in to morphologically distinguishable structures, facilitated the studies on intracellular trafficking. In subsequent studies, the dynamics of MBP mRNA transport have been studied using a combination of cell culture, microinjection and confocal microscopy. Fluorescently labeled exogenous MBP mRNA microinjected into the cell body of cultured oligodendrocytes is rapidly assembled into granules within minutes after injection that can be visualized in living cells using confocal fluorescent microscopy (Ainger et al.,

1993). This suggested that exogenous mRNA assembles into pre-packaged ribonucleoprotein complexes in the cell body. In fact, the granule assembly may be prerequisite for further trafficking of mRNAs. Since, both endogenous MBP mRNA and microinjected exogenous MBP mRNA displays punctuated distribution, these mRNAs are termed "RNA granules". After assembly in to RNA granules, mRNAs are transported in an anterograde direction along the processes and eventually localize to the myelin compartment (Ainger et al., 1993). A variety of different mRNAs have been visualized as granules in other systems, including fibroblasts, neurons, Drosophila embryos and Xenopus oocytes. Recent Co-localization studies and biochemical purification of RNA granules from embryonic and adult brain indicate that these granules consist of multiple RNA copies and contain many proteins including components of the translational machinery (Kanai et al., 2004; Elvira et al., 2006).

Therefore, in eukaryotes a general RNA granule hypothesis may state that multiple mRNA copies are assembled with components of RNA binding proteins (RBPs) and the protein synthetic machinery into complexes that can be visualized as RNA granules by a variety of imaging techniques, and that intracellular trafficking of RNA granules is determined by cis-acting elements in the RNA molecules contained in each granule and by transacting factors within the cell.

Cis-acting elements

Cis-acting elements have been identified in 3'UTR by deleting various regions of MBP mRNA and analyzing the subcellular distribution of the injected RNA in the cell body. These studies revealed, a 21 nucleotide cis-acting element termed the RTS (RNA trafficking sequence), is necessary and sufficient for RNA transport along the oligodendrocyte processes. When the RTS is deleted, RNA is not transported along the processes or localized to myelin compartment, but are remained in the cell body. When RTS is inserted to heterologous non transported RNAs such as GFP, it causes the chimeric RNA to be transported to processes, but not localized to the myelin compartment indicating that the RTS is necessary and sufficient for RNA transport (Ainger et al., 1997). A second element that has been termed the ‘RNA localization signal’ (RLS), is required for localization of RNA to the myelin compartment. When RLS is deleted, the RNA forms granules that are transported along the processes but not localized to the myelin compartment (Ainger et al., 1997). Insertion of the RLS

causes RNA to move from processes to myelin compartment. Therefore, RTS and RLS are necessary in MBP mRNA and sufficient to target any RNA to the myelin compartment. Bio computational studies predicted that, the region containing the RLS in 3'UTR has a significant secondary structure that is conserved in all MBP mRNAs from different species (Ainger et al., 1997). Thus, the postulated function of the RLS is to anchor MBP mRNA in the myelin compartment. RTS homologous sequences are found in a verity of different mRNAs that are known to be transported in other systems as shown in table 1, Ainger et al., 1997). This means that studies of RNA trafficking in oligodendrocytes are probably relevant in order to understand other systems.

Table 1. RTS homology in transported mRNAs

Species	mRNA	Region	RTS sequence
Rat	MBP	3′UTR	GCCAAGGAGCCAGAGAGCAUG
Mouse	MBP	3′UTR	GCCAAGGAGCCAGAGAGCAUG
Human	MBP RTS1	3′UTR	GCCA**U**GGAG**G**CA**C**A**C**AGC UG
Human	MBP RTS2	3′UTR	GC**UGCA**GAG**A**CAGAGAG**G**A**C**G
Rat	MOBP81A	3′UTR	**A**CC**CCC**GAG**A**CA**C**AGAGCAUG
Rat	GFAP	ORF	GCCAAGGAGCC**CACC**A**AAC**UG
Mouse	protamine 2	3′UTR	GCCAAGGAGCCA**CGAGAUC**UG
		consensus	GCCAAGGAGCCAGAGAGCAUG

Transacting factors

The RTS identification as a cis-acting trafficking element in MBP mRNA and possibly other mRNAs, suggested the existence of cellular transacting factors that recognize the RTS. Trans acting factors that bind to the RTS have been isolated by RNA affinity chromatography. In these experiments RTS containing RNA oligonucleotides were coupled to beads and the beads incubated with protein extracts. Analysis of the bound proteins were performed by mass spectrometry and Western blots. By following this approach a number of A/B type hnRNP proteins include hnRNP A2 (Hoek et al., 1998), hnRNP A3(Ma et al., 2002) and CBF-A (Raju et al., 2008) were identified as RTS binding factors. Since chronologically hnRNP A2 was the first to be identified to interact with RTS, the RTS element is also referred to as A2 Response Element (A2RE). When hnRNPA2 expression is suppressed by antisense oligonucleotides treatment, transport of microinjected MBP mRNA or RTS containing RNA is inhibited to oligodendrocyte processes. Similarly, when hnRNP CBFA is suppressed by RNA interference, endogenous MBP mRNA retained in the cell body excluding in

cytoplasmic processes (Raju et al., 2008). Interestingly co localization studies indicate that, hnRNPA2, CBF-A and MBP mRNA are in the same granules.

Translation regulation

As mentioned earlier, eukaryotic mRNAs follow defined intracellular trafficking pathways from their sites of transcription to their sites of translation (Dreyfuss et al., 2002). Localization of a specific mRNA to a particular sub cellular compartment provides mechanism to maximize the translation of the encoded protein in the region where they are needed most and to minimize ectopic expression elsewhere in the cell. This implies that translational regulation is an integral part of intracellular RNA trafficking pathway. Translation is thought to be repressed during mRNA transport and activated once the RNA is localized or has reached its destination. Several proteins involved in suppression of translation during RNA transport have been identified in mammalian cells (Hüttelmaier et al., 2005). However, molecular mechanism of translation regulation during RNA trafficking has not been determined. There is evidence that translation of specific mRNAs can be regulated by trans cting RNA binding proteins that bind to cis-acting sequences in the RNAs. For instance, the cis acting RTS element is known to enhance translational efficiency of reporter constructs by several fold in vitro and in vivo (Kwon et al., 1999). Furthermore, using dicistronic RNA, the RTS specifically enhances cap-dependent translation without affecting internal ribosome entry site (IRES)-dependent translation. Subsequent studies indicated that hnRNP E1 is recruited to MBP mRNA/RTS RNA granules, presumably to suppress the translation, while transit to myelin compartment (Kosturko et al., 2006). In this study it was revealed that hnRNP E1 binds hnRNP A2 in vitro and is co localized with hnRNP A2 and RTS RNA in granules in oligodendrocyte processes. Furthermore, it was shown that hnRNP E1 inhibits translation of RTS containing RNA in vitro and in vivo possibly by binding to hnRNPA2. Later studies indicated that phosphorylation of hnRNPA2 by Fyn kinase relieves translational inhibition of MBP mRNA after reaching the myelin compartment. Neuronal adhesion molecule L1 binding to oligodendrocytes results in Fyn activation, which leads to an increase in hnRNP A2 phosphorylation (White et al., 2008).

Insights into mRNA transport in neurons

Neurons are highly polarized cells and contain specialized sub cellular domains that usually include an axon and multiple dendrites, which extend great distances from the

cell body. Axons and dendrites have specialized functions that require different sub set of proteins for their respective functions. That implies demand for multiple proteins to be asymmetrically distributed and targeted to correct sub cellular domains. One strategy used by neurons to meet this challenge is differential RNA localization and local protein synthesis. Many RNAs are localized along the length of axon and axon terminals. Within the axon, local protein synthesis is thought to be involved in growth and growth cone mediated axon guidance in response environmental cues. RNA localization is also prominent feature in dendrites. Dendrites contain many small protrusions termed dendritic spines onto each of which a single synapse is formed. Dendrites and dendritic spines have acquired lot of interest in terms of RNA localization as local protein synthesis at these sites is thought to modulate synaptic functions in learning and memory. Therefore, local protein synthesis provides growth cones and synapses with the capacity to autonomously regulate their structure and function.

In neurons, the hypothesis that mRNAs are transported, localized, and locally translated in dendrites originates from evidence obtained by electron microscopy that polyribosomes are present in dendrites (Bodian et al., 1965) and in the neck of spine heads of granular neurons (Steward and Levy.,1982). Later studies using radioactive uridine precursors, demonstrated that RNAs were transported to dendrites of cultured hippocampal neurons (Davis et al., 1987). Subsequent in situ hybridization studies revealed the presence of specific mRNAs encoding cytoskeletal, integral membrane proteins in dendritic layers and at postsynaptic densities of hippocampal neurons in vivo and in vitro. These transcripts include microtubule-associated protein 2 (MAP2) mRNA (Garner et al., 1988; Kleiman et al., 1990), the α subunit of Ca2/calmodulin dependent protein kinase II (CaMKIIα) mRNA (Miyashiro et al., 1994), brain-derived neurotrophic factor (BDNF) mRNA, activity-regulated cytoskeleton-associated protein (Arc) mRNA (Lyford et al., 1995). Dynamic translocation of endogenous mRNAs in neuronal processes was then described in live cells, using the membrane-permeable nucleic acid stain SYTO 14 (Knowles et al., 1996). Later on translocation of specific mRNA to neuronal processes was studied in cultured hippocampal neurons by tethering a green fluorescent protein (GFP) to the 3'-untranslated region (3'UTR) of the αCaMKII mRNA with the MS2 bacteriophage tagging system (Rook et al., 2000). Unbiased approaches to amplify mRNAs from purified dendritic and/or synaptic compartments (Miyashiro et al., 1994; Tian et al., 1999; Moccia et al., 2003; Sung et

al., 2004) have identified hundreds of localized mRNAs. Nowadays, different approaches are being used to show that RNA granules are mobile structures with anterograde, retrograde, and oscillatory movements.

Now it is well established that many RNAs are targeted to both axons and dendrites (for review, see Bramham, C.R. and Wells, 2007). However, the molecular mechanism by which specific mRNAs are transported is not fully understood. The present working model on how neuronal mRNAs are transported summarized in figure 5 and include the following steps. (1) recognition of RNA cis-acting elements by RNA-binding proteins (transacting factors) in the nucleus (2) export of mRNA/mRNPs from the nucleus to the cytoplasm (3) association of additional factors including molecular motors to form functional RNA granules (4) transport of RNA granules on cytoskeletal structures (5) localization and/or anchoring of RNA to its destination and (6) translational de repression of the localized mRNAs by specific signals.

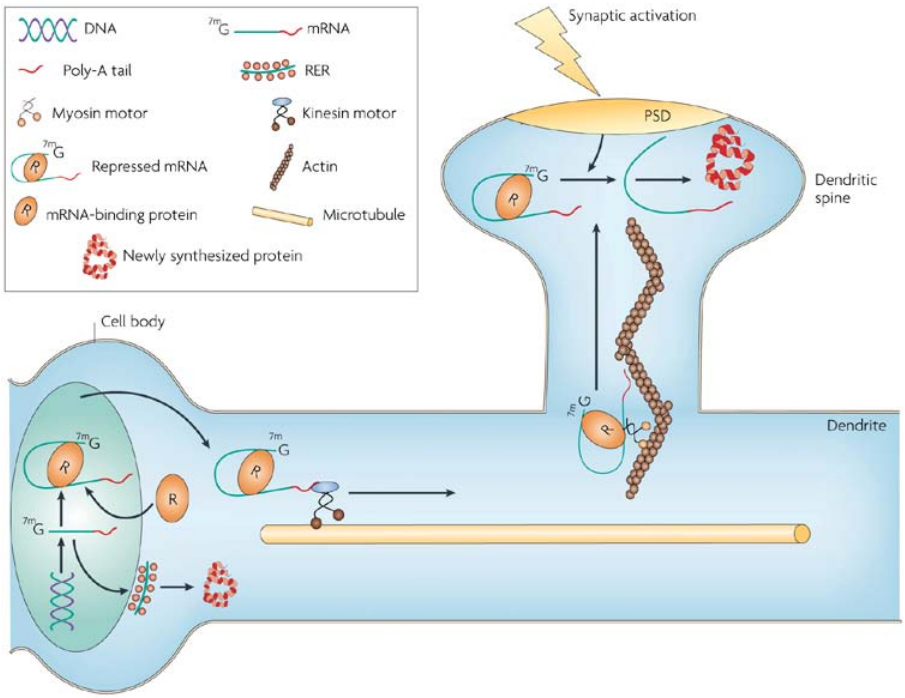

Figure 5. Working model for mRNA transport and local translation in neurons. Transport of mRNAs constitutively present in the dendritic domain is increased in response to stimuli at specific synapses. Transcribed mRNAs are first recognized in the nucleus by RNA-binding proteins (R) and exported to the cytoplasm. RNPs are then packaged with additional factors into granules that are transported into the dendrite by kinesin motors on microtubules. Following synaptic stimulation, the granules are dispersed and the mRNA is anchored/ localized to spines by the actin-based myosin motor proteins. Translation of localized mRNA is activated at the synaptic compartment by de repression of RNA binding protein. 7mG, 7-methyl-guanosine; PSD, postsynaptic density (Adapted from Bramham, C.R. and Wells, 2007)

It is currently believed that targeting of mRNAs to dendrites mainly depends on the presence of dendritic targeting elements (DTEs) or Zipcodes within RNA. As I mentioned in the introduction these RNA sequences are normally found in the 3'UTR of mRNAs. Recognition of cis-acting elements by trans acting RNA-binding proteins results in the formation of RNP complexes competent for their transport on the cytoskeleton. Deletion and expression studies have led to the identification of functional DTEs. These RNA elements are necessary and sufficient to mediate the dendritic transport of reporter transcripts. DTEs from different transcripts are quite different in length, sequence, number, and position ranging from 54 nucleotides in the case of β-actin mRNA to around 1200 nucleotides for the αCaMKII transcript. This diversity in the zipcode sequences/structures may parallel the complexity of the mechanisms of mRNA transport and translation. So far, no clear consensus "cis acting" sequence or structure has been identified. Given the complexity of DTEs, it is predicted that DTEs are recognized by RNA-binding proteins through complex structural elements rather than via linear sequences.

Composition of neuronal transported RNA granules

Similarly to the MBP mRNA granules in oligodendrocytes, it is believed that localized mRNAs travel as large mRNP complexes or RNA granules that may be associated with ribosomes. Two different studies focused on the molecular characterization of mRNPs that were isolated from neural tissue to understand their roles in RNA transport and the nature of the molecules that regulate transport, translation, stability. The first study utilized the interaction of transport RNPs with the conventional kinesin KIF5 to isolate large RNase-sensitive granules and there by restricting the characterization to only transport RNPs (Kanai et al., 2004). Overall 42 proteins were identified by mass spectometry as well as at least two dendritically targeted mRNAs encoding CaMKIIα and Arc. The identified proteins include known regulators of mRNA transport (FMR1, FXR1, FXR2, Pur α, Pur β, staufen), protein synthesis (EF-1α, eIF2α, eIF2β, eIF2γ, Hsp70, ribosomal protein L3), RNA helicases (DDX1, DDX3, DDX5) and several hnRNP proteins (hnRNP-A/B or CBF-A, hnRNP-A0, hnRNP-A1, hnRNP-D, hnRNP-U). Interestingly, these granules lack the cytoplasmic-polyadenylation-element binding protein 1 (CPEB1) a protein that was earlier shown to bind to CaMKIIα mRNA. CPEB1 was shown to participate in its localization into dendrites and regulate its translation (Wu, L. et al., 1998; Huang et al., 2003). In addition, these granules do not contain hnRNP A2, a protein identified in neuronal RNA transport granules in

dendrites of cultured neurons (Shan et al., 2003). β-actin mRNA and its trans acting protein ZBP1 are also absent in these RNA granules, which is consistent with the notion that the pool of RNPs is heterogeneous.

The second study applied subcellular fractionation to isolate a fraction that was enriched in RNPs from developing rat brains and subjected this fraction to proteomic analysis (Elvira et al., 2006). In contrast to the KIF5 granule, these large granules obtained in this study contained ZBP1 and mRNA encoding β-actin, but not CaMKIIα. In spite of these differences, the two RNP preparations share many common components, including several heterogeneous hnRNPs, SYNCRIP, FMRP, Pur-α, Pur-β, Staufen and DEAD (Asp-Glu-Ala-Asp) BOX 1, DDX3 DDX5. Altogether these studies indicated that there are core components of transport RNPs, which are always required and that additional RNA binding proteins are added or removed based on brain region or developmental stage.

Activity regulated transport

Live-cell imaging techniques using fluorescently labeled RNA or RNA binding proteins revealed that a fraction of mRNA granules displayed either rapid anterograde or retrograde movements that are likely to be dependent on microtubules (Sánchez-Carbente et al., 2008). In neurons, these movements can be altered by neuronal activity. In fact neuronal activity is known to increase the extent of mRNA transport in dendrites (Elvira et al., 2006; Kanai et al., 2004) indicating that mRNA transport is highly regulated. Neuronal activity resulted in an increase in the number of dendritically localized CaMKIIα mRNA containing granules (Rook et al., 2000). Neuronal activity also lead to re localize mRNAs from Staufen1containing RNA granules to actively translating polyribosomes (Krichevsky and Kosik, 2001). However, the mechanism of translocation to the polyribosomes is not known. Similarly, RNG105 protein dissociates from RNA granules in dendrites following treatment of the cells with BDNF and this mechanism is correlated with the translation of a reporter transcript (Shiina et al., 2005). Neuronal depolarization is also consistent with increased ZBP1-containing granules into dendrites and it is dependent on N-Methyl-D-aspartate (NMDA) receptor activation (Tiruchinapalli et al., 2003). In vivo, synaptic activation strongly up regulates Arc gene expression and the recruitment of newly synthesized corresponding transcripts to activated synapses requires NMDA receptors (Steward & Worley., 2001). The RNA binding protein TLS is translocated to dendritic spines by

mGluR5 activation and regulates spine morphology (Fujii et al., 2005). Similarly, activation of metabotropic glutamate receptors (mGluRs) promotes the localization of mRNAs encoding the AMPA receptor subunits GluR1 and GluR2 into dendrites (Grooms et al., 2006). Long-term potentiation (LTP) is a persistent increase in signal transmission between two neurons that results from high frequency stimulation. LTP induction in the CA1 region of hippocampal slices causes translocation of polyribosomes in to spines after 2 hours (Ostroff et al., 2002). Elevated levels of CaMKIIα mRNA were detected in synaptodendrosomes following LTP induction in the dentate gyrus of awake rats (Havik et al., 2003). Altogether these results indicate that that synaptic activity leads to increased levels of dendritic mRNA transport and a prolonged increase in dendritic protein synthesis.

Translational regulation of dendritic mRNAs

RNA transport granules have been reported to lack essential translational components and to be unable to incorporate radioactive amino acids indicating that they are not translationally competent (Krichevsky and Kosik., 2001). It is believed that RNA-binding proteins bind to specific cis-elements and are capable of negatively regulating mRNA translation (Wells et al., 2006). One such example for this type is how ZBP1 binds to β-actin mRNA, as mentioned earlier binding occur co transcriptionally and this mechanism probably keeps mRNA in a translationally dormant state. In the cytoplasm ZBP1 could be dissociated from the mRNA by src kinase nediated phosphorylation, therefore allowing β-actin mRNA to be translated (Huttelmaier et al., 2005). Likewise, the RNA binding protein RNG105 is a translational repressor that has been found associated with neuronal RNA granules (Shiina et al 2005). Other RNA binding proteins such as polyadenylation element-binding protein (CPEB) and FMRP are regulators of specific mRNA translation in neurons (Huang et al., 2002; Laggerbauer et al., 2001).

The translation of an mRNA into a corresponding protein involves three sequential. steps, normally initiation, elongation, and termination. Regulation can occur at any of these steps, but initiation is usually rate limiting and thus often a primary target for regulation. Protein synthesis is critical for persistent synaptic modifications like LTP and LTD (long-term depression) (Kindler et al ., 2005; Bramham, C.R. and Wells., 2007). Several signaling pathways have been reported in neurons that modulate translation by targeting translation initiation factor eIF4E. eIF4E is a subunit of the

eIF4F complex, binds to the 5'cap of mRNAs and promotes recruitment of the 43S preinitiation complex (Kapp and Lorsch., 2004). Cap-dependent translation in dendrites and postsynaptic domains can be stimulated by two receptor coupled kinase pathways. ERK (extracellular signal-regulated) signaling leads to phosphorylation of eIF4E and mTOR (mammalian target of rapamycin) signaling which leads to phosphorylation of eIF4E-BP (eIF4E-binding protein) and release of eIF4E, which eventually becomes available for cap binding (Raught et al. 2000; Aakalu et al., 2001; Richter et al., 2005). In support of this, key components of the mTOR pathway, such as mTOR, eIF4E, and eIF4E-BP, have been identified in postsynaptic domains (Tang et al., 2002). Synapto-dendritic protein synthesis that affects synaptic strength is also controlled at the level of peptide chain elongation. eEF2 is a GTP-binding protein that facilitates the translocation of peptidyl-tRNAs from the A-site to the P-site on the ribosome. Phosphorylation of eEF2 results in overall decrease in global protein synthesis, while increasing enhanced translation of two dendritic mRNAs such as Arc and CamKIIα mRNA that are important in synaptic plasticity (Nairn et al., 2001). NMDA treatment of synaptodendrosomes enhances eEF2 phosphorylation and suppresses global protein synthesis while increasing CaMKIIα expression (Scheetz et al., 2000).

Translational control of gene expression in synapto-dendritic domains is also controlled by microRNAs. miR-134, a brain-specific miRNA that is found in the synapto dendritic compartment and negatively regulates dendritic spine morphogenesis in cultured hippocampal neurons by repressing translation of LIMK1 mRNA. BDNF application removes miR134 mediated repression of LIMK1 translation and promotes spine morphogenesis (Schratt et al., 2006).

In summary, translational repression appears to be important during RNA transport to prevent ectopic expression in neurons. Appropriately controlled protein synthesis at the synapse is important neuronal excitation-inhibition balance and therefore, it may be essential to have multiple controls in place to keep local translation in the silent mode until a valid and correct signal is received. The nature of such a signal, and the mechanism by which it is transduced to the local translational machinery is a very challenging task to understand (for review, see Kindler et al., 2005; Bramham, C.R. and Wells., 2007).

RNA transport and translation during spermatogenesis

In mammalian testis highly specialized control of gene expression both at the transcriptional and post transcriptional levels exists during spermatogenesis, which ensures the production of mature sperm and the maintenance of the species (reviewed in Sassone-Cors et al., 2002). One of the unique phenomena in male germ cell development is the condensation of sperm chromatin. During late spermiogenesis, the chromatin of the haploid spermatids highly condenses and in fact all transcription activity stops (Söderström et al., 1981; Söderström et al., 1976). For this reason, the mRNAs coding proteins required in late spermatids are transcribed in the earlier stage of spermatogenesis and thought to be stored in special organelles termed “chromatoid body” (CB) in the cytoplasm and translated when spermatids need them. Consistent with this view, delayed translation of many mRNAs occurs in these post-meiotic cells (Tanaka et al., 2005; Kleene et al., 1993; Kleene et al., 1996).

Chromatoid body

The term chromatoid body (CB) is derived from the fact that this cytoplasmic structure is strongly stained by basic dyes similar to chromosomes and nucleoli. In mammals, the CB is observed in the cytoplasm from early spermatocytes to late spermatids and its shape is usually spherical. As spermatids develop, the CB assumes various shapes and its size gradually diminishes. First it appears adjacent to the nuclear envelope where it is juxtaposed to nuclear pore complexes (juxtanuclear cytoplasm) and then it moves towards the cytoplasm at the base of the flagellum. High resolution electron microscopy studies revealed material continuities exists between the nucleus and the CB as if the CB can selectively collect nuclear material (Söderström et al., 1976; Parvinen et al., 1979). The CB has never been biochemically isolated with high purity so far, hence to date there is no biochemical charecterization of this structure. However histochemical studies indicate CB contains RNA and many proteins (Kotaja et al., 2007). Although its function remains difficult to understand, the CB may be involved in temporary RNA storage and processing due to the fact it contains various RNA-binding proteins and RNA (Fujiwara et al., 1994; Toyooka et al., 2000; Tsai-Morris et al., 2004; Nguyen Chi et al., 2009; Kotaja et al., 2006; Saunders et al., 1992). Therefore it is believed that CB plays a crucial role in post-transcriptional control during spermatogenesis.

KIF17b is a testis-specific kinesin that shuttles between cytoplasmic and nuclear compartments and it is concentrated in the CBs of round spermatids. Based on its functional properties, it is suggested to transport of specific RNPs components from the nucleus to the CB (Kotaja et al., 2006). However in elongating spermatids, CBs are functionally transformed, presumably in response to developmental cue calls, releasing multiple components including such as the microRNA biding protein MIWI (Shang et al., 2010) and RNA transcripts which are then localized for translation.

Protamines are nuclear proteins which are expressed in mature sperm. Protamines are believed to replace histones in late phase of spermatogenesis and are essential for sperm head chromatin condensation (for review, see Sassone-Corsi et al., 2002). Interestingly, protamine 1 and 2 expression seems to be regulated at the mRNA levels. Protamine 1 and 2 mRNAs transcripts are known to be stored for 2 to 7 days before their active translation (Balhorn et al., 1984; Kleene et al., 1984). Previous studies using transgenic mice demonstrated the importance of the 3'UTR sequence of protamine 1 mRNA which is also necessary for translational repression (Lee et al.,1995). Furthermore premature translation of protamine 1 mRNA leads to early nuclear condensation, resulting in male infertility (Lee et al.,1995). Many proteins which bind to specific sequences in 3'UTR of protamine mRNAs have been identified (Dadoune et al, 2003), however the molecular mechanisms that control temporary storage and regulation of translation remain unclear. Less is known about protamine 2 mRNA. However this transcript contain conserved RTS sequence in its 3'UTR (Carson et al., 1997). Therefore it is possible that RTS mediated transport to localize protamine 2 mRNA is important during spermatogenesis.

AIMS

The overall goal of this thesis work has been to dissect how hnRNP proteins interact with the UTR of mRNA transcripts to regulate their transport and localization mechanism in different mammalian cell types. By focusing on the A/B type hnRNP protein CBF-A, the following specific aims were addressed.

1. To evaluate whether CBF-A binds RTS sequences on the MBP mRNA and functions as novel transacting factor to facilitate MBP mRNA trafficking to myelin compartment of oligodendrocytes.

2. To determine whether and how the transacting role of CBF-A is required for transport of dendritic mRNAs in neurons and it is activity dependent.

3. To evaluate the role of CBF-A in spatially and temporally regulated mRNAs during spermatogenesis.

RESULTS AND CONCLUSIONS

Paper 1

In the first paper included in the thesis we were interested in identifying novel transacting factors implicated in MBP mRNA biogenesis in oligodendrocytes. As I mentioned in the introduction, the RTS cis-acting element plays an important role and in oligodendrocytes it is required for the localization of MBP mRNA to the myelin compartment. In order to identify novel transacting factors, we set out to perform RNA affinity chromatography. In these experiments biotinylated RNA oligonucleotides containing the MBP mRNA RTS found in 3'UTR are coupled to the streptavidin coated beads and incubated with Hela cells nuclear, cytoplasmic and cytoskeletal protein extracts. RNA oligonucleotides containing scramble versions of the MBP mRNA RTS sequence were coupled to the beads and used as a control. In all cases bound proteins were analyzed by Coomassie staining and Western blotting. Using antibodies against core hnRNP proteins we discovered that both CBF-A and A2 coprecipitated with wtRTS. Sub cellular fractions of Hela cells and protein-protein interaction studies revealed that hnRNP A2, A3, and CBF-A are physically associated in RNA dependent manner. From these results, we conclude that CBF A is in multiprotein complexes where it specifically associates with the MBP mRNA RTS element.

Association of CBFA with wtRTS raises the question as to whether CBFA binds RTS directly or indirectly. In order to test this possibility, we performed electrophoretic mobility shift assay (EMSA). In this assay, radioactive labeled RTS containing RNA oligonucleotides were incubated with the purified recombinant CBF-A along with hnRNP A2 or A3 and used as positive controls. The resulting RNA-protein complexes were resolved by native gel electrophoresis. We found that CBF-A retarded the electrophoretic mobility of wtRTS containing RNA oligonucleotides. We concluded that CBF-A directly binds to wild type RTS sequences and the specificity of the binding was confirmed by competition studies.

Analysis of steady state expression on immunoblots showed that CBF-A is ubiquitously expressed in mouse tissues. Immunostaining of mouse brain sections with the anti-CBF-A antibody followed by confocal microscopy revealed that CBF-A is expressed all over in the forebrain, showing distinctive nuclear signals. Interestingly, a significant fraction of CBF-A was also found outside the cell nucleus in discrete

particles which are reminiscent of transported RNA granules in oligodendrocytes and neurons, also granules displayed different sizes and signal intensities depending on the brain regions analyzed. Double Immunolabelling studies revealed that most of the CBF-A positive granules were found in close proximity to CNPase, suggesting a specialized cytoplasmic function for CBF-A, presumably in processes emanating from the cell body. Similarly, CBF-A positive granules were also revealed in cytoplasmic processes of primary oligodendrocytes, astrocytes, and neurons obtained by in vitro differentiation of fetal rat and adult mouse neural stem cells. In all these cases, CBF-A positive granules were revealed along the microtubule-rich processes.

The above findings that CBF-A binds directly to RTS and is present in discrete granules in the processes of oligodendrocytes, prompted us to test whether CBF-A colocalizes with MBP mRNA transport granules along the cytoplasmic processes. For this reason, we have used oligodendrocyte precursor cells called oli-neu, which can be terminally differentiated into myelin producing oligodendrocytes (Jung et al., 1995). Differentiated oli-neu cells were subjected to fluorescent in situ hybridization (FISH) using antisense MBP mRNA probes followed by immunostaining with anti-CBF-A antibodies. Confocal microscopy revealed that CBF-A positive granules distributed along oligodendrocytes processes colocalize with MBP mRNA in granular structures. Quantification of fluorescent intensity of individual granules and statistical analysis indicate that approximately 90% of granules were simulntaneously positive for both CBF-A and MBP mRNA and showed linear correlations between the CBF-A and MBP mRNA signals. Similar results were obtained in the case of hnRNP A2. Triple labeling experiments revealed that more than 80% of the granules were positive for MBP mRNA, CBF-A and A2. From these results we conclude that cytoplasmic CBF-A colocalize with the RNA transport granules that include MBP mRNA and hnRNPA2 in oligodendrocyte processes. These results were further supported by immunoprecipitation and qRT-PCR experiments. Immunoprecipitation performed on total oli-neu cells using anti-CBFA antibodies precipitated hnRNPA2 in the absence of RNAse treatment. On the contrary, hnRNPA2 was not precipitated when cell extracts were treated with RNAse A before immunoprecipitation. These results suggest that CBF-A and hnRNP A2 are part of the same RNA-containing complexes, but their association is dependent on RNA. Furthermore qRT-PCR analysis on immunoprecipitated samples indicate that significant enrichment was observed for MBP mRNA isoforms compared to SOX genes encoding transcription factors. From

the above results we conclude that in differentiating oli-neu cells a significant proportion of CBF-A is specifically associated with the MBP mRNA, presumably as part of MBP mRNA granules.

If CBF-A binds RTS sequence and associates with MBP mRNA and a part of transported MBP mRNA granules CBF-A must be implicated in transport and localization of MBP mRNA to myelin compartment. In support of this hypothesis, we discovered that CBF-A gene knock down by siRNA affected the distribution of MBP mRNA in oligodendrocytes. We found that MBP mRNA was mainly detected in the cell body, being excluded from the processes.
Finally, cryoimmunoelectron microscopy using anti CBF-A antibody revealed that CBF-A was detected in different cellular compartments of oligodendrocytes. As expected for hnRNP proteins, CBF-A is detected in the nucleus. In the cytoplasm CBF is found in distinctive granular structures presumably RNA transport granules that are associated with cytoskeleton molecules both with microtubules and microfilamemnets. CBF-A labeling is also detected in concentric multilamellar structures which resemble formation of myelin membranes. Taken altogether these observations indicate that CBF-A accompany mRNA from cell nucleus to myelin compartment in oligodendrocytes.

In summary, the above findings suggest that CBF-A is novel transacting factor required for cytoplasmic MBP mRNA transport and localization to myelin compartment of oligodendrocytes.

Paper 2

In the second paper we analyzed the transacting role of CBF-A in transport and localization of dendritic mRNAs in neurons.

In neurons, many different RNAs are targeted to dendrites and synapses where local expression of the encoded proteins mediates synaptic plasticity during learning and memory (for review, see Kindler et al 2005; Bramham, C.R. and Wells, D.G. 2007). However, the mechanisms by which these mRNAs are targeted to dendrites and synapses are not fully understood. Previous evidence that CBF-A is a component of isolated RNA granules from embryonic and adult brain (Kanai et al., 2004: Elvira et al., 2006) and our findings presented in paper1 (Raju et al., 2008) that in the brain CBF-A

present in discrete cytoplasmic granular structures led us to believe, CBF-A may act as transacting factor for certain mRNAs that are transported to dendrites and synapses. In support of this view, confocal and electron microscopy revealed that CBF-A localizes to discrete granules along dentries and in pre and post synaptic terminals. Furthermore, immunoelectron microscopy on brain sections revealed that CBF-A is present in interchromosomal spaces (ICS) and in the perichromatin area where active transcription takes place (Fakan and Puvion 1980), indicating that CBF-A may present in nascent transcripts. Since CBF-A was also found associated with dense structures at the nuclear pores, presumably mRNPs, in transit to the cytoplasm, we concluded that in neurons CBF-A accompanies mRNA to the cytoplasm.

The presence of CBF-A at distal dendrites and synaptic sites raised the question as to whether CBF-A associates with mRNAs that are targeted to dendrites and synapses. For this reason, we have purified synaptosomal fractions and performed RNA immunoprecipitation (RIP) using anti CBF-A antibodies. The precipitated RNA was then analyzed by RT-PCR. We revealed that significant enrichment for dendritically targeted Arc, BDNF and CaMKIIα mRNAs in comparison to α-tubulin and GAPDH mRNA. These results indicate that in synaptosomal preparations CBF-A associates with Arc, BDNF and CaMKIIα mRNAs. RTS and RTS-like sequences are reported to be present in different mRNAs (Carson et al., 1997) and dendritically transported mRNAs in the 3' UTRs of CaMKIIα and Arc mRNAs (Gao et al., 2008). In the present paper, we also report such RTS-like motif exists in the 3'UTR of BDNF mRNA and it is likely to be functional. Our previous findings that CBF-A binds directly to the RTS element from MBP mRNA, prompted us to test whether CBF-A binds RTS like sequences in dentritically targeted Arc, BDNF and CaMKIIα mRNAs. RNA affinity chromatography using RNA oligonucleotides containing RTS like sequences from Arc, BDNF, and CaMKIIα mRNAs was performed on total brain extracts. We found that CBF-A coprecipitated with the wtRTS like sequences, but not with control oligonucleotides containing scrambled RTS sequences. The direct binding to RTS like sequences from Arc, BDNF, and CaMKIIα mRNAs and the specificity of the binding was further confirmed by EMSA. Therefore we conclude CBF-A specifically binds RTS like sequences present in dendritically targeted Arc, BDNF, and CaMKIIα mRNAs.

We next wanted to study if CBF-A colocalizes with transport RNA granules in dendrites. Immuno-FISH experiments on cultured hippocampal neurons revealed that

CBF-A co localizes with Arc, BDNF, and CaMKIIα mRNAs in granules along the dendrites. Quantification of fluorescent intensity of individual granules demonstrated linear correlation between CBF-A and above mentioned mRNAs. Distribution of exogenous EGFP tagged CBF-A is also somewhat similar to endogenous one and shown to be incorporated into the cytoplasmic discrete granules along the dendrites. Furthermore, exogenous CBF-A granules partly colocalizes with endogenous CaMKIIα mRNA as revealed by in situ hybridization. Time lapse analysis on hippocampal neurons transiently expressing EGFP-CBF-A, found that a major fraction of EGFP CBF-A positive granules were immobile or stationary and a fraction of granules exhibited an oscillatory movement. A subset of EGFP CBF-A positive granules displayed a single direction motion either anterograde or retrograde, with a calculated speed of 0.028-0.13 μm/sec similar to that measured for Staufen 1, DDX3 and CaMKIIα mRNA-containing granules (Kohrmann et al. 1999; Elvira et al, 2006; Rook et al, 2000).

In mammalian neurons many RNAs are present in dendrites, but some of these transcripts were shown to undergo activity regulated transport (Bramham, C.R. and Wells, D.G. 2007; Sánchez-Carbente., et al 2008). Synaptic activation in vivo strongly up regulates Arc gene expression and the recruitment of newly synthesized Arc transcripts to activated synapses requires NMDA receptors (Steward & Worley., 2001). Similarly, activity-dependent localization of BDNF (Tongiorgi et al., 1997) and CaMKIIα (Mori et al., 2000; Rook et al., 2000) transcripts has been reported in dendrites of cultured neurons. Neuronal depolarization also led to increase in the ZBP1-containing granules into dendrites, a mechanism which is also dependent on NMDA receptor activation (Tiruchinapalli et al., 2003). Consistent with this view, treatment of cultured hippocampal neurons with the NMDA and AMPA agonists resulted in an increase in the levels of CBF- A containing granules in dendrites. Furthermore, the same treatment also led to increased levels of Arc, BDNF, and CaMKIIα mRNAs as revealed by qRT-PCR. Increased transcript levels also correlated with increased amounts of transcripts coprecipitating with CBF-A. Finally, CBF-A gene knock down by siRNA caused the decreased mRNA levels (at least 2 fold drops) in dendrites. We conclude that CBF-A is required for targeting RTS containing mRNAs to dendrites.

Paper 3

In the third paper we studied the role of CBF-A in the trafficking of spatially and temporally regulated transcripts such as the RTS containing protamine 2 mRNA during spermatogenesis.

To assess the intracellular distribution of protamine 2 mRNA we carried out fluorescence in situ hybridization (FISH) on cryosections of mouse testis using antisense probe followed by confocal microscopy. These results show that in elongating spermatids, protamine 2 mRNA localized all over in the cytoplasm displaying diffuse staining, where as in round spermatids it is preferentially detected in the nucleus and in perinuclear structures, being excluded from the cytoplasm. These perinuclear structures were later confirmed to be chromatoid bodies as they are positive for the marker protein MVH (mouse vasa homolog) by immunostaining. These results indicate that in round spermatids following transcription, protamine 2 mRNA is targeted and temporarily stored in chromatoid bodies and then dispersed into the cytosol of elongating spermatids.

Similarly, we also evaluated the cellular distribution of CBF-A in spermatogenic cells by immunostaining followed by confocal microscopy. We found that CBF-A is expressed in spermatogenic cells at all developmental stages. CBF-A predominantly localized to nuclei and cytosol in round spermatids and elongating spermatids respectively. When the sections were subjected to antigen retrieval, CBF-A also accumulate into perinuclear structures similar to chromatoid bodies. Squash preparations of seminiferous tubules to dissociate testicular cells into single intact cells that retain the morphology (Page et al., 1998) followed by double immunolabelling of these specimens showed that a considerable fraction of CBF-A localizes in chromatoid body. Consistent with the previous evidence that CBF-A and hnRNP A2 are part of the same complex (Raju et al.,2008) similar degree of overlap was revealed after double immunostaining with antibodies to CBF-A and hnRNP A2. From these results, we conclude that in round spermatids CBF-A is abundant in the cell nucleus and accumulates in chromatoid bodies whereas in elongating spermatids CBF-A is preferentially found in the cytoplasm. Taken altogether these observations suggest that

during spermatogenesis CBF-A is transported to chromatoid bodies and it is later on dispersed in the cytoplasm presumably with haploid mRNAs.

Since CBF-A and protamine 2 mRNA exhibit somewhat similar distribution in spermatogenic cells, we next wanted to determine whether CBF-A associates or interacts with the protamine 2 mRNA. For this reason, we prepared nuclear and cytoplasmic fractions from mouse testes and subjected to RNA immunoprecipitation analysis (RIP). In both cases CBF-A antibodies co-precipitated CBF-A and hnRNP A2, but did not precipitate MVH/VASA and the piRNA-binding protein MIWI which also localizes to chromatoid bodies and is involved in germline development (Lin et al., 2007; Unhavaithaya et al., 2009). However these co-precipitations were impaired when nuclear and cytoplasmic extracts were treated with RNase A prior to the immunoprecipitation assays indicating that CBF-A is part of specific RNP complexes. The RIP analysis from cytoplasmic fractions further demonstrated that the protamine 2 mRNA transcript was coprecipitated with CBF-A antibodies, but was not detected upon RNase treatment performed prior to the immunoprecipitation step. On the contrary α-tubulin and clusterin mRNAs were only marginally detected in the immunoprecipitated fractions independently of the RNase treatment. Densitometric quantifications showed a considerable two fold increase in the amount of protamine 2 mRNA precipitated with CBF-A in comparison with α-tubulin and clusterin mRNAs. These observations suggest that CBF-A associates with protamine 2 mRNA in cytoplasmic fraction.

Previously it was predicted that protamine 2 mRNA contains RTS like sequence in its 3'UTR, which is highly similar to the RTS sequences found in the MBP mRNA. Injection of a reporter plasmid containing the protamine 2 mRNA RTS like sequence induced transport of the reporter RNA to distal sites of oligodendrocytes (Ainger et al., 1997) indicating that protamine 2 mRNA RTS is probably functional. In our studies, RNA affinity chromatography and EMSA revealed that CBFA specifically binds protamine 2 mRNA RTS which is consistent with the RTS binding property of CBF-A in oligodendrocytes and neurons (Raju et al., 2008; 2011).

Since protamine 2 mRNA and CBF-A are dispersed in the cytoplasm of elongating spermatids and they are part of the same RNP complex, we next examined whether CBF-A engaged in protamine 2 mRNA translation. For this purpose, cytoplasmic extracts from testis were subjected to ultracentrifugation on sucrose gradient to separate

RNPs, monosomes and polyribosomes. We found that CBF-A co sedimented with protamine 2 mRNA in the polyribosome rich fractions, where as EDTA treatment which disrupts translationally active polyribosomes, both CBF-A and protamine 2 mRNA were mostly recovered in the RNP fraction. Interestingly analysis of the fractions with the antibodies to both CBF-A isoforms p37 and p42 showed that it is the p42 that co sediments with polyribosomes. However, upon EDTA treatment, both CBF-A splice variants were recovered at the top of the gradient cosedimenting with RNP and monosomes rich fractions. Based on above observations, we conclude that both CBF-A variants associates with protamine 2 mRNA, but only the p42 isoform accompany the transcript to translationally active polyribosomes. In support of this view, when cytoplasmic lysate were incubated with immobilized 7-methyl-GTP-cap analog beads (m7GTP beads), the p42 variant was pulled down together with the eukaryotic initiation factor eIF4E, MIWI and hnRNP A2. These results altogether suggest that CBF-A bound to the RTS in the 3'UTR of protamine 2 mRNA may be important for mRNA targeting to translationally active polyribosomes.

GENERAL DISCUSSION

Here we provide evidence that the hnRNP CBF-A specifically binds the conserved RTS cis-acting elements which are in the 3' UTR of mRNA transcripts that are expressed in several cell types. In oligondendrocytes, CBF-A binds to the MBP mRNA RTS, in hippocampal neurons we show specific interaction with the RTSs found in ARC, CaMKIIa and BDNF mRNAs and in testicular cells, we found a direct interaction with the RTS in the protamine 2 mRNA. These interactions were verified in vitro and in living cells. These findings and evidence that CBF-A gene silencing lead to an alteration in the distribution of these transported mRNA transcripts indicates that CBF-A has a general role as transacting factor in RTS-mediated mRNA transport and localization. Therefore we propose that CBF-A is rather critical for the establishment of asymmetric mRNA and protein distribution, a mechanism that is required for specialized cellular function.

Our data also support the view that CBF-A binds RTS-containing transcripts already in the cell nucleus and remains associated with these transcripts also in the cytoplasm. A close look at the distribution of CBF-A in the nucleus of brain cells by electron microscopy shows that CBF-A is excluded from dense chromatin and CBF-A is present in nascent pre-mRNA. These observations and evidence that CBF-A does not seem to interact with transcripts that do not contain RTS sequences (α-tubulin and GAPDH mRNAs) de facto provides a mechanism that sorts transport-competent mRNA molecules at an early stage during the mRNA biogenesis pathway during cotranscriptional assembly of pre-mRNPs. An interesting possibility is that CBF-A binding to RTSs lead to remodeling of the 3'UTR and this mechanism facilitates the establishment of a specific RNP configuration required for cytoplasmic transport and localization.

Previously it was reported that hnRNP A2 binds RTS sequences in the 3'UTR of different mRNAs. Based on those findings it was suggested that hnRNP A2 is a transacting factor required for transport of MBP mRNA and dendritic mRNAs to myelin compartment and synapses, respectively. Our results show evidence that CBF-A and hnRNP A2 are present in the same transported mRNA granules and they are part of the same complex in an RNA-dependent manner. At this stage it is not clear why two transacting factors bind the same element and are required for mRNA trafficking. In any case the fact that they are part of the same transported granules suggests that CBF-

A and hnRNP A2 cooperate for efficient mRNA transport and localization to subcellular compartments. An interesting possibility is that their roles are dependent on the local environment and the set of interactions required for the establishment of trafficking intermediates (Carson and Barbarese, 2005). In support of this view, a similar situation occurs in the case of β-actin mRNA. ZBP1 and ZBP2 bind to the same zipcode sequence and cooperate to localize beta-actin mRNA (Pan et al., 2007).

Evidence from independent laboratories suggested that synaptic activity leads to increased levels of dendritic mRNA transport and a prolonged increase in dendritic protein synthesis. This observation led to the hypothesis that mRNA transport and local protein synthesis in dendrites is probably important for synaptic plasticity related to LTP (see for review Bramham and Wells, 2007). In our work we found that treatment of hippocampal neurons with NMDA and AMPA agonists resulted in an increase in the levels of CBF-A at dendrites. Interestingly this increase correlated with increased levels of CaMKIIα mRNA granules as also revealed by Rook et al., 2000. These findings and evidence that binding of CBF-A to dendritic transcripts is enhanced upon postsynaptic receptor activation, indicate that the transacting role of CBF-A in neuronal mRNA transport and localization is activity-dependent. Furthermore, we found that CBF-A gene knockdown by siRNA resulted in a drop in the levels of dendritic transcripts including CaMKIIα. In a mutant mouse model expressing a truncated version of the CaMKIIα gene lacking the 3' UTR which contains the RTS element, the RNA was restricted to the cell body. The same mouse model showed reduction in late-phase long-term potentiation (LTP) and impaired spatial memory (Miller et al., 2002). Increased levels of CaMKIIα mRNA were detected in synaptodendrosomes following LTP induction in the dentate gyrus of awake rats (Havik et al., 2003). We conclude that CBF-A and cis-acting RTS in CaMKIIα mRNA may be important for transport and localization upon synaptic activity and a similar scenario is likely to occur for Arc and BDNF mRNA during synaptic activity.

The fact that CBF-A recognizes the functional RTS element of protamine 2 mRNA and follows the transcript through the entire mRNA biogenesis pathway in testicular cells suggest that mRNA trafficking is a general mechanism to control gene expression for specialized cellular function. Here the interesting finding is that the well characterized CBF-A isoforms, p37 and p42, appear to have different roles in protamine 2 mRNA biogenesis. We find that in round spermatids, where protamine 2 mRNA is kept in a

translationally inhibited form, both variants accompany the transcript to chromatoid body. On the other hand in elongating spermatids where protamine 2 mRNA is translated only the largest CBF-A isoform, p42, remains associated with the transcript and accompanies it to translationally active polyribosome. Evidence that the p42 variant but not the p37 one binds to the 5' cap structure of the mRNA suggests that p42 may facilitate targeting of the protamine 2 mRNA transcript to polyribosomes. We speculate that p37 could be important to maintain a translationally inactive form. Taken altogether these observations point to the possibility that the differential functions of the CBF-A variants may be important to regulate protamine 2 mRNA trafficking in the context of spermatogenesis.

In conclusion our results indicate that CBF-A acts as a general transacting factor in RTS-mediated mRNA transport and localization and these mechanisms are important for specialized cellular function by promoting asymmetric mRNA and protein distribution.

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REFERENCES

Aakalu, G., Smith, W.B., Nguyen, N., Jiang, C., and Schuman, E.M. 2001. Dynamic visualization of local protein synthesis in hippocampal neurons. Neuron 30(2): 489-502.

Alberts, B. 2008. Molecular biology of the cell. Garland Science, Newyork

Aguilera, A. 2005. Cotranscriptional mRNP assembly: from the DNA to the nuclear pore. Current Opinion in Cell Biology 17(3): 242-250.

Ainger, K., Avossa, D., Diana, A.S., Barry, C., Barbarese, E., and Carson, J.H. 1997. Transport and localization elements in myelin basic protein mRNA. J Cell Biol 138(5): 1077-1087.

Ainger, K., Avossa, D., Morgan, F., Hill, S.J., Barry, C., Barbarese, E., and Carson, J.H. 1993. Transport and localization of exogenous myelin basic protein mRNA microinjected into oligodendrocytes. J Cell Biol 123(2): 431-441.

AmurUmarjee, S., Schonmann, V., and Campagnoni, A.T. 1997. Neuronal regulation of myelin basic protein mRNA translocation in oligodendrocytes is mediated by platelet-derived growth factor. Developmental Neuroscience 19(2): 143-151.

An, J.J., Gharami, K., Liao, G.-Y., Woo, N.H., Lau, A.G., Vanevski, F., Torre, E.R., Jones, K.R., Feng, Y., Lu, B. et al. 2008. Distinct Role of Long 3' UTR BDNF mRNA in Spine Morphology and Synaptic Plasticity in Hippocampal Neurons. Cell 134(1): 175-187.

Aronov, S., Aranda, G., Behar, L., and Ginzburg, I. 2001. Axonal tau mRNA localization coincides with tau protein in living neuronal cells and depends on axonal targeting signal. J Neurosci 21(17): 6577-6587.

Aronov, S., Gelin-Licht, R., Zipor, G., Haim, L., Safran, E., and Gerst, J.E. 2007. mRNAs Encoding Polarity and Exocytosis Factors Are Cotransported with the Cortical Endoplasmic Reticulum to the Incipient Bud in Saccharomyces cerevisiae. Mol Cell Biol 27(9): 3441-3455.

Balhorn, R., Weston, S., Thomas, C., and Wyrobek, A.J. 1984. DNA packaging in mouse spermatids : Synthesis of protamine variants and four transition proteins. Experimental Cell Research 150(2): 298-308.

Barbarese, E. 1991. Spatial distribution of myelin basic protein mRNA and polypeptide in Quaking oligodendrocytes in culture. Vol 29, pp. 271-281. Wiley Subscription Services, Inc., A Wiley Company.

Bassell, G. and Singer, R.H. 1997. mRNA and cytoskeletal filaments. Current Opinion in Cell Biology 9(1): 109-115.

Bassell, G.J. and Kelic, S. 2004. Binding proteins for mRNA localization and local translation, and their dysfunction in genetic neurological disease. Curr Opin Neurobiol 14(5): 574-581.

Bassell, G.J., Zhang, H.L., Byrd, A.L., Femino, A.M., Singer, R.H., Taneja, K.L., Lifshitz, L.M., Herman, I.M., and Kosik, K.S. 1998. Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. Journal of Neuroscience 18(1): 251-265.

Beach, D.L., Salmon, E.D., and Bloom, K. 1999. Localization and anchoring of mRNA in budding yeast. Current Biology 9(11): 569-578, S561.

Bemark, M., Olsson, H., Heinegard, D., and Leanderson, T. 1998. Purification and characterization of a protein binding to the SP6 Kappa promoter - A potential role for CArG-box binding factor-A in Kappa transcription. Journal of Biological Chemistry 273(30): 18881-18890.

Benjamins, J.A. and Morell, P. 1978. Proteins of myelin and their metabolism. Neurochemical Research 3(2): 137-174.

Bodian, D. 1965. A Suggestive Relationship of Nerve Cell Rna with Specific Synaptic Sites. Proceedings of the National Academy of Sciences of the United States of America 53(2): 418

Bramham, C.R. and Wells, D.G. 2007. Dendritic mRNA: transport, translation and function. Nat Rev Neurosci 8(10): 776-789.

Burd, C.G. and Dreyfuss, G. 1994. Conserved Structures and Diversity of Functions of Rna-Binding Proteins. Science 265(5172): 615-621.

Carson, J.H. and Barbarese, E. 2005. Systems analysis of RNA trafficking in neural cells. Biol Cell 97(1): 51-62.

Carson, J.H., Cui, H., and Barbarese, E. 2001. The balance of power in RNA trafficking. Current Opinion in Neurobiology 11(5): 558-563.

Carson, J.H., Gao, Y., Tatavarty, V., Levin, M.K., Korza, G., Francone, V.P., Kosturko, L.D., Maggipinto, M.J., and Barbarese, E. 2008. Multiplexed RNA trafficking in oligodendrocytes and neurons. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 1779(8): 453-458.

Carson, J.H., Kwon, S., and Barbarese, E. 1998. RNA trafficking in myelinating cells. Current Opinion in Neurobiology 8(5): 607-612.

Carson, J.H., Worboys, K., Ainger, K., and Barbarese, E. 1997. Translocation of myelin basic protein mRNA in oligodendrocytes requires microtubules and kinesin. Cell Motility and the Cytoskeleton 38(4): 318-328.

Chabot, B., Blanchette, M., Lapierre, I., and LaBranche, H. 1997. An intron element modulating 5' splice site selection in the hnRNP A1 pre-mRNA interacts with hnRNP A1. Molecular and Cellular Biology 17(4): 1776-1786.

Chartrand, P., Meng, X.H., Huttelmaier, S., Donato, D., and Singer, R.H. 2002. Asymmetric Sorting of Ash1p in Yeast Results from Inhibition of Translation by Localization Elements in the mRNA. Molecular Cell 10(6): 1319-1330.

Chartrand, P., Meng, X.H., Singer, R.H., and Long, R.M. 1999. Structural elements required for the localization of ASH1 mRNA and of a green fluorescent protein reporter particle in vivo. Current Biology 9(6): 333-338.

Chennathukuzhi, V., Morales, C.R., El-Alfy, M., and Hecht, N.B. 2003. The kinesin KIF17b and RNA-binding protein TB-RBP transport specific cAMP-responsive element modulator-regulated mRNAs in male germ cells. Proceedings of the National Academy of Sciences of the United States of America 100(26): 15566-15571.

Cole, C.N. and Hammell, C.M. 1998. Nucleocytoplasmic transport: Driving and directing transport. Current Biology 8(11): R368-R372.

Colman, D.R., Kreibich, G., Frey, A.B., and Sabatini, D.D. 1982. Synthesis and Incorporation of Myelin Polypeptides into Cns Myelin. Journal of Cell Biology 95(2): 598-608.

Condeelis, J. and Singer, R.H. 2005. How and why does beta-actin mRNA target? Biology of the Cell 97(1): 97-110.

Cooper, T.A., Wan, L., and Dreyfuss, G. 2009. RNA and Disease. Cell 136(4):777-793.

Cote, C.A., Gautreau, D., Denegre, J.M., Kress, T.L., Terry, N.A., and Mowry, K.L. 1999. A Xenopus protein related to hnRNP I has a role in cytoplasmic RNA localization. Molecular Cell 4(3): 431-437.

Czaplinski, K. and Singer, R.H. 2006. Pathways for mRNA localization in the cytoplasm. Trends in Biochemical Sciences 31(12): 687-693.

Dadoune, J.-P. 2003. Expression of mammalian spermatozoal nucleoproteins. Microscopy Research and Technique 61(1): 56-75.

Daneholt, B. 1997. A look at messenger RNP moving through the nuclear pore. Cell 88(5): 585-588.

Daneholt, B. 2001. Assembly and transport of a premessenger RNP particle. Proceedings of the National Academy of Sciences 98(13): 7012-7017.

Dean, J.L.E., Sully, G., Wait, R., Rawlinson, L., Clark, A.R., and Saklatvala, J. 2002. Identification of a novel AU-rich-element-binding protein which is related to AUF1. Biochemical Journal 366: 709-719.

Deshler, J.O., Highett, M.I., and Schnapp, B.J. 1997. Localization of Xenopus Vg1 mRNA by vera protein and the endoplasmic reticulum. Science 276(5315): 1128-1131.

Dictenberg, J.B., Swanger, S.A., Antar, L.N., Singer, R.H., and Bassell, G.J. 2008. A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. Dev Cell 14(6): 926-939.

Dreyfuss, G., Kim, V.N., and Kataoka, N. 2002. Messenger-RNA-binding proteins and the messages they carry. Nature Reviews Molecular Cell Biology 3(3): 195-205.

Dreyfuss, G., Matunis, M.J., Pinol-Roma, S., and Burd, C.G. 1993. hnRNP proteins and the biogenesis of mRNA. Annu Rev Biochem 62: 289-321.

Eberwine, J., Belt, B., Kacharmina, J.E., and Miyashiro, K. 2002. Analysis of subcellularly localized mRNAs using in situ hybridization, mRNA amplification, and expression profiling. Neurochemical Research 27(10): 1065-1077.

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. Characterization of an RNA granule from developing brain. Mol Cell Proteomics 5(4): 635-651.

Eom, T., Antar, L.N., Singer, R.H., and Bassell, G.J. 2003. Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. Journal of Neuroscience 23(32): 10433-10444.

Ephrussi, A., Dickinson, L.K., and Lehmann, R. 1991. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell 66(1): 37-50.

Fakan, S. and Puvion, E. 1980. The ultrastructural visualization of nucleolar and extranucleolar RNA synthesis and distribution. Int Rev Cytol 65: 255-299.

Farina, K.L., Huttelmaier, S., Musunuru, K., Darnell, R., and Singer, R.H. 2003. Two ZBP1 KH domains facilitate beta-actin mRNA localization, granule formation, and cytoskeletal attachment. Journal of Cell Biology 160(1): 77-87.

Ferrandon, D., Elphick, L., Nüsslein-Volhard, C., and St Johnston, D. 1994. Staufen protein associates with the 3'UTR of bicoid mRNA to form particles that move in a microtubule-dependent manner. Cell 79(7): 1221-1232.

Ferrandon, D., Koch, I., Westhof, E., and Nusslein-Volhard, C. 1997. RNA-RNA interaction is required for the formation of specific bicoid mRNA 3[prime] UTR-STAUFEN ribonucleoprotein particles. EMBO J 16(7): 1751-1758.

Fujii, R., Okabe, S., Urushido, T., Inoue, K., Yoshimura, A., Tachibana, T., Nishikawa, T., Hicks, G.G., and Takumi, T. 2005. The RNA Binding Protein TLS Is Translocated to Dendritic Spines by mGluR5 Activation and Regulates Spine Morphology. Current Biology 15(6): 587-593.

Fujiwara, Y., Komiya, T., Kawabata, H., Sato, M., Fujimoto, H., Furusawa, M., and Noce, T. 1994. Isolation of a Dead-Family Protein Gene That Encodes a Murine Homolog of Drosophila-Vasa and Its Specific Expression in Germ-Cell Lineage. Proceedings of the National Academy of Sciences of the United States of America 91(25): 12258-12262.

Gao, Y., Tatavarty, V., Korza, G., Levin, M.K., and Carson, J.H. 2008. Multiplexed Dendritic Targeting of {alpha} Calcium Calmodulin-dependent Protein Kinase II, Neurogranin, and Activity-regulated Cytoskeleton-associated Protein RNAs by the A2 Pathway. Mol Biol Cell 19(5): 2311-2327.

Garcia-Ladona, F.J., Huss, Y., Frey, P., and Ghandour, M.S. 1997. Oligodendrocytes express different isoforms of β-amyloid precursor protein in chemically defined cell culture conditions: In situ hybridization and immunocytochemical detection. Journal of Neuroscience Research 50(1): 50-61.

Garner, C.C., Tucker, R.P., and Matus, A. 1988. Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. Nature 336(6200): 674-677.

Gavis, E.R. and Lehmann, R. 1992. Localization of Nanos Rna Controls Embryonic Polarity. Cell 71(2): 301-313.

Ghandour, M.S. and Skoff, R.P. 1991. Double-labeling in situ hybridization analysis of mRNAs for carbonic anhydrase II and myelin basic protein: expression in developing cultured glial cells. Glia 4(1): 1-10.

Giorgi, C., Yeo, G.W., Stone, M.E., Katz, D.B., Burge, C., Turrigiano, G., and Moore, M.J. 2007. The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. Cell 130(1): 179-191.

Glanzer, J., Miyashiro, K.Y., Sul, J.Y., Barrett, L., Belt, B., Haydon, P., and Eberwine, J. 2005. RNA splicing capability of live neuronal dendrites. Proc Natl Acad Sci U S A 102(46): 16859-16864.

Gonzalez, I., Buonomo, S.B.C., Nasmyth, K., and von Ahsen, U. 1999. ASH1 mRNA localization in yeast involves multiple secondary structural elementsand Ash1 protein translation. Current Biology 9(6): 337-340.

Grooms, S.Y., Noh, K.-M., Regis, R., Bassell, G.J., Bryan, M.K., Carroll, R.C., and Zukin, R.S. 2006. Activity Bidirectionally Regulates AMPA Receptor mRNA Abundance in Dendrites of Hippocampal Neurons. The Journal of Neuroscience 26(32): 8339-8351.

Groth, A., Rocha, W., Verreault, A., and Almouzni, G. 2007. Chromatin challenges during DNA replication and repair. Cell 128(4): 721-733.

Gu, W., Pan, F., Zhang, H.L., Bassell, G.J., and Singer, R.H. 2002. A predominantly nuclear protein affecting cytoplasmic localization of beta-actin mRNA in fibroblasts and neurons. Journal of Cell Biology 156(1): 41-51.

Hachet, O. and Ephrussi, A. 2001. Drosophila Y14 shuttles to the posterior of the oocyte and is required for oskar mRNA transport. Current biology : CB 11(21): 1666-1674.

Hachet, O. and Ephrussi, A. 2004. Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization. Nature 428(6986): 959-963.

Hay, B., Jan, L.Y., and Jan, Y.N. 1988. A protein component of Drosophila polar granules is encoded by vasa and has extensive sequence similarity to ATP-dependent helicases. Cell 55(4): 577-587.

Hilleren, P., McCarthy, T., Rosbash, M., Parker, R., and Jensen, T.H. 2001. Quality control of mRNA 3 '-end processing is linked to the nuclear exosome. Nature 413(6855): 538-542.

Hoek, K.S., Kidd, G.J., Carson, J.H., and Smith, R. 1998. hnRNP A2 selectively binds the cytoplasmic transport sequence of myelin basic protein mRNA. Biochemistry 37(19): 7021-7029.

Holz, A., Schaeren-Wiemers, N., Schaefer, C., Pott, U., Colello, R., and Schwab, M. 1996. Molecular and developmental characterization of novel cDNAs of the myelin-associated/oligodendrocytic basic protein. The Journal of Neuroscience 16(2): 467-477.

Huang, F., Chotiner, J.K., and Steward, O. 2007. Actin Polymerization and ERK Phosphorylation Are Required for Arc/Arg3.1 mRNA Targeting to Activated Synaptic Sites on Dendrites. The Journal of Neuroscience 27(34): 9054-9067.

Huang, Y.S., Carson, J.H., Barbarese, E., and Richter, J.D. 2003. Facilitation of dendritic mRNA transport by CPEB. Genes Dev 17(5): 638-653.

Huang, Y.-S., Jung, M.-Y., Sarkissian, M., and Richter, J.D. 2002. N-methyl-D-aspartate receptor signaling results in Aurora kinase-catalyzed CPEB phosphorylation and [alpha]CaMKII mRNA polyadenylation at synapses. EMBO J 21(9): 2139-2148.

Huttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X.H., Bassell, G.J., Condeelis, J., and Singer, R.H. 2005. Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature 438(7067): 512-515.

Håvik, B., Røkke, H., Bårdsen, K., Davanger, S., and Bramham, C.R. 2003. Bursts of high-frequency stimulation trigger rapid delivery of pre-existing α-CaMKII mRNA to synapses: a mechanism in dendritic protein synthesis during long-term potentiation in adult awake rats. European Journal of Neuroscience 17(12): 2679-2689.

Jambhekar, A. and Derisi, J.L. 2007. Cis-acting determinants of asymmetric, cytoplasmic RNA transport. Rna-a Publication of the Rna Society 13(5): 625-642.

Jeffery, W.R., Tomlinson, C.R., and Brodeur, R.D. 1983. Localization of Actin Messenger-Rna during Early Ascidian Development. Developmental Biology 99(2): 408-417.

Jenny, A., Hachet, O., Zavorszky, P., Cyrklaff, A., Weston, M.D., Johnston, D.S., Erdelyi, M., and Ephrussi, A. 2006. A translation-independent role of oskar RNA in early Drosophila oogenesis. Development 133(15): 2827-2833.

Johnstone, O. and Lasko, P. 2001. Translational regulation and RNA localization in Drosophila oocytes and embryos. Annual Review of Genetics 35: 365-406.

Jung, M., Kramer, E., Grzenkowski, M., Tang, K., Blakemore, W., Aguzzi, A., Khazaie, K., Chlichlia, K., Vonblankenfeld, G., Kettenmann, H. et al. 1995. Lines of Murine Oligodendroglial Precursor Cells Immortalized by an Activated Neu Tyrosine Kinase Show Distinct Degrees of Interaction with Axons in-Vitro and in-Vivo. European Journal of Neuroscience 7(6): 1245-1265.

Kamada, S. and Miwa, T. 1992. A Protein-Binding to Carg Box Motifs and to Single-Stranded-DNA Functions as a Transcriptional Repressor. Gene 119(2): 229-236.

Kapp, L.D. and Lorsch, J.R. 2004. The molecular mechanics of eukaryotic translation. Annual Review of Biochemistry 73: 657-704.

Katahira, J., Straszer, K., Podtelejnikov, A., Mann, M., Jung, J.U., and Hurt, E. 1999. The Mex67p-mediated nuclear mRNA export pathway is conserved from yeast to human. EMBO J 18(9): 2593-2609.

Kelley, R.L. 1993. Initial Organization of the Drosophila Dorsoventral Axis Depends on an Rna-Binding Protein Encoded by the Squid Gene. Genes & Development 7(6): 948-960.

Kiebler, M.A., Hemraj, I.A., Verkade, P., Kohrmann, M., Fortes, P., Marion, R.M., Ortin, J., and Dotti, C.G. 1999. The mammalian Staufen protein localizes to the somatodendritic domain of cultured hippocampal neurons: Implications for its involvement in mRNA transport. Journal of Neuroscience 19(1): 288-297.

Kiledjian, M., DeMaria, C., Brewer, G., and Novick, K. 1997. Identification of AUF1 (heterogeneous nuclear ribonucleoprotein D) as a component of the alpha-globin mRNA stability complex. Mol Cell Biol 17(8): 4870-4876.

Kimha, J., Smith, J.L., and Macdonald, P.M. 1991. Oskar Messenger-Rna Is Localized to the Posterior Pole of the Drosophila Oocyte. Cell 66(1): 23-34.

Kindler, S., Wang, H.D., Richter, D., and Tiedge, H. 2005. RNA transport and local control of translation. Annual Review of Cell and Developmental Biology 21: 223-245.

King, M.L., Messitt, T.J., and Mowry, K.L. 2005. Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. Biology of the Cell 97(1): 19-33.

Kislauskis, E.H., Zhu, X., and Singer, R.H. 1994. Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. The Journal of Cell Biology 127(2): 441-451.

Kleene, K.C. 1993. Multiple Controls over the Efficiency of Translation of the mRNAs Encoding Transition Proteins, Protamines, and the Mitochondrial Capsule Selenoprotein in Late Spermatids in Mice. Developmental Biology 159(2): 720-731.

Kleene, K.C. 1996. Patterns of translational regulation in the mammalian testis. Molecular Reproduction and Development 43(2): 268-281.

Kleene, K.C., Distel, R.J., and Hecht, N.B. 1984. Translational regulation and deadenylation of a protamine mRNA during spermiogenesis in the mouse. Developmental Biology 105(1): 71-79.

Kleiman, R., Banker, G., and Steward, O. 1990. Differential subcellular localization of particular mRNAs in hippocampal neurons in culture. Neuron 5(6): 821-830.

Knowles, R.B., Sabry, J.H., Martone, M.E., Deerinck, T.J., Ellisman, M.H., Bassell, G.J., and Kosik, K.S. 1996. Translocation of RNA granules in living neurons. J Neurosci 16(24): 7812-7820.

Koenig, E. 1965. Synthetic Mechanisms in Axon .2. Rna in Myelin-Free Axons of Cat. Journal of Neurochemistry 12(5): 357-&

Kosturko, L.D., Maggipinto, M.J., Korza, G., Lee, J.W., Carson, J.H., and Barbarese, E. 2006. Heterogeneous Nuclear Ribonucleoprotein (hnRNP) E1 Binds to hnRNP A2 and Inhibits Translation of A2 Response Element mRNAs. Mol Biol Cell 17(8): 3521-3533.

Kotaja, N., Lin, H., Parvinen, M., and Sassone-Corsi, P. 2006. Interplay of PIWI/Argonaute protein MIWI and kinesin KIF17b in chromatoid bodies of male germ cells. J Cell Sci 119(13): 2819-2825.

Kotaja, N. and Sassone-Corsi, P. 2007. The chromatoid body: a germ-cell-specific RNA-processing centre. Nat Rev Mol Cell Biol 8(1): 85-90.

Krecic, A.M. and Swanson, M.S. 1999. hnRNP complexes: composition, structure, and function. Current Opinion in Cell Biology 11(3): 363-371.

Krichevsky, A.M. and Kosik, K.S. 2001. Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. Neuron 32(4): 683-696.

Kristensson, K., Holmes, K.V., Duchala, C.S., Zeller, N.K., Lazzarini, R.A., and Dubois-Dalcq, M. 1986. Increased levels of myelin basic protein transcripts gene in virus-induced demyelination. Nature 322(6079): 544-547.

Kroll, T.T., Zhao, W.M., Jiang, C., and Huber, P.W. 2002. A homolog of FBP2/KSRP binds to localized mRNAs in Xenopus oocytes. Development 129(24): 5609-5619.

Kwon, S., Barbarese, E., and Carson, J.H. 1999. The Cis-Acting RNA Trafficking Signal from Myelin Basic Protein mRNA and Its Cognate Trans-Acting Ligand Hnrnp A2 Enhance CaP-Dependent Translation. The Journal of Cell Biology 147(2): 247-256.

Kyburz, A., Friedlein, A., Langen, H., and Keller, W. 2006. Direct interactions between subunits of CPSF and the U2 snRNP contribute to the coupling of pre-mRNA 3 ' end processing and splicing. Molecular Cell 23(2): 195-205.

Laggerbauer, B., Ostareck, D., Keidel, E.-M., Ostareck-Lederer, A., and Fischer, U. 2001. Evidence that fragile X mental retardation protein is a negative regulator of translation. Human Molecular Genetics 10(4): 329-338.

Lall, S., Francis-Lang, H., Flament, A., Norvell, A., Schupbach, T., and Ish-Horowicz, D. 1999. Squid hnRNP protein promotes apical cytoplasmic transport and localization of Drosophila pair-rule transcripts. Cell 98(2): 171-180.

Landry, C.F., Watson, J.B., Kashima, T., and Campagnoni, A.T. 1994. Cellular influences on RNA sorting in neurons and glia: an in situ hybridization histochemical study. Brain Res Mol Brain Res 27(1): 1-11.

Laursen, L.S., Chan, C.W., and ffrench-Constant, C. Translation of myelin basic protein mRNA in oligodendrocytes is regulated by integrin activation and hnRNP-K. The Journal of Cell Biology 192(5): 797-811.

Lawrence, J.B. and Singer, R.H. 1986. Intracellular-Localization of Messenger-Rnas for Cytoskeletal Proteins. Cell 45(3): 407-415.

Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T.R., Tomancak, P., and Krause, H.M. 2007. Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell 131(1): 174-187.

Lee, K., Haugen, H.S., Clegg, C.H., and Braun, R.E. 1995. Premature translation of protamine 1 mRNA causes precocious nuclear condensation and arrests spermatid differentiation in mice. Proceedings of the National Academy of Sciences 92(26): 12451-12455.

Lewis, R.A., Kress, T.L., Cote, C.A., Gautreau, D., Rokop, M.E., and Mowry, K.L. 2004. Conserved and clustered RNA recognition sequences are a critical feature of signals directing RNA localization in Xenopus oocytes. Mechanisms of Development 121(1): 101-109.

Li, B., Carey, M., and Workman, J.L. 2007. The role of chromatin during transcription. Cell 128(4): 707-719.

Liang, S., Hitomi, M., Hu, Y.H., Liu, Y., and Tartakoff, A.M. 1996. A DEAD-box-family protein is required for nucleocytoplasmic transport of yeast mRNA. Molecular and Cellular Biology 16(9): 5139-5146.

Lin, A.C. and Holt, C.E. 2007. Local translation and directional steering in axons. Embo Journal 26(16): 3729-3736.

Loflin, P., Chen, C.Y.A., and Shyu, A.B. 1999. Unraveling a cytoplasmic role for hnRNP D in the in vivo mRNA destabilization directed by the AU-rich element. Genes & Development 13(14): 1884-1897.

Long, R.M., Singer, R.H., Meng, X., Gonzalez, I., Nasmyth, K., and Jansen, R.-P. 1997. Mating Type Switching in Yeast Controlled by Asymmetric Localization of ASH1 mRNA. Science 277(5324): 383-387.

LoPresti, P., Szuchet, S., Papasozomenos, S.C., Zinkowski, R.P., and Binder, L.I. 1995. Functional implications for the microtubule-associated protein tau: localization in oligodendrocytes. Proceedings of the National Academy of Sciences 92(22): 10369-10373.

Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A., and Worley, P.F. 1995. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 14(2): 433-445.

Ma, A.S.W., Moran-Jones, K., Shan, J., Munro, T.P., Snee, M.J., Hoek, K.S., and Smith, R. 2002. Heterogeneous nuclear ribonucleoprotein A3, a novel RNA trafficking response element-binding protein. Journal of Biological Chemistry 277(20): 18010-18020.

Macchi, P., Kroening, S., Palacios, I.M., Baldassa, S., Grunewald, B., Ambrosino, C., Goetze, B., Lupas, A., St Johnston, D., and Kiebler, M. 2003. Barentsz, a new component of the Staufen-containing ribonucleoprotein particles in mammalian cells, interacts with Staufen in an RNA-dependent manner. J Neurosci 23(13): 5778-5788.

Macdonald, P.M. and Kerr, K. 1997. Redundant RNA recognition events in bicoid mRNA localization. Rna-a Publication of the Rna Society 3(12): 1413-1420.

Macdonald, P.M., Kerr, K., Smith, J.L., and Leask, A. 1993. RNA regulatory element BLE1 directs the early steps of bicoid mRNA localization. Development 118(4): 1233-1243.

Macdonald, P.M. and Struhl, G. 1988. Cis- acting sequences responsible for anterior localization of bicoid mRNA in Drosophila embryos. Nature 336(6199): 595-598.

Martin, K.C. and Ephrussi, A. 2009. mRNA Localization: Gene Expression in the Spatial Dimension. Cell 136(4): 719-730.

Martin, K.C. and Zukin, R.S. 2006. RNA trafficking and local protein synthesis in dendrites: An overview. Journal of Neuroscience 26(27): 7131-7134.

Matsuzaki, F., Ohshiro, T., Ikeshima-Kataoka, H., and Izumi, H. 1998. miranda localizes staufen and prospero asymmetrically in mitotic neuroblasts and epithelial cells in early Drosophila embryogenesis. Development 125(20): 4089-4098.

Matunis, E.L., Kelley, R., and Dreyfuss, G. 1994. Essential Role for a Heterogeneous Nuclear Ribonucleoprotein (Hnrnp) in Oogenesis - Hrp40 Is Absent from the Germ-Line in the Dorsoventral Mutant Squid. Proceedings of the National Academy of Sciences of the United States of America 91(7): 2781-2784.

Mayeda, A. and Krainer, A.R. 1992. Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. Cell 68(2): 365-375.

Melton, D.A. 1987. Translocation of a localized maternal mRNA to the vegetal pole of Xenopus oocytes. Nature 328(6125): 80-82.

Messitt, T.J., Gagnon, J.A., Kreiling, J.A., Pratt, C.A., Yoon, Y.J., and Mowry, K.L. 2008. Multiple Kinesin Motors Coordinate Cytoplasmic RNA Transport on a Subpopulation of Microtubules in Xenopus Oocytes. Developmental cell 15(3): 426-436.

Michael, W.M., Choi, M.Y., and Dreyfuss, G. 1995. A Nuclear Export Signal in Hnrnp A1 - a Signal-Mediated, Temperature-Dependent Nuclear-Protein Export Pathway. Cell 83(3): 415-422.

Michael, W.M., Eder, P.S., and Dreyfuss, G. 1997. The K nuclear shuttling domain: A novel signal for nuclear import and nuclear export in the hnRNP K protein. Embo Journal 16(12): 3587-3598.

Mikheev, A.M., Mikheev, S.A., Zhang, Y., Aebersold, R., and Zarbl, H. 2000. CArG binding factor A (CBF-A) is involved in transcriptional regulation of the rat Ha-ras promoter. Nucleic Acids Research 28(19): 3762-3770.

Miller, S., Yasuda, M., Coats, J.K., Jones, Y., Martone, M.E., and Mayford, M. 2002. Disruption of dendritic translation of CaMKII alpha impairs stabilization of synaptic plasticity and memory consolidation. Neuron 36(3): 507-519.

Miyashiro, K.Y., Beckel-Mitchener, A., Purk, T.P., Becker, K.G., Barret, T., Liu, L., Carbonetto, S., Weiler, I.J., Greenough, W.T., and Eberwine, J. 2003. RNA Cargoes Associating with FMRP Reveal Deficits in Cellular Functioning in Fmr1 Null Mice. Neuron 37(3): 417-431.

Moccia, R., Chen, D., Lyles, V., Kapuya, E., E, Y., Kalachikov, S., Spahn, C.M.T., Frank, J., Kandel, E.R., Barad, M. et al. 2003. An Unbiased cDNA Library Prepared from Isolated Aplysia Sensory Neuron Processes Is Enriched for Cytoskeletal and Translational mRNAs. The Journal of Neuroscience 23(28): 9409-9417.

Mohr, S.E., Dillon, S.T., and Boswell, R.E. 2001. The RNA-binding protein Tsunagi interacts with Mago Nashi to establish polarity and localize oskar mRNA during Drosophila oogenesis. Genes Dev 15(21): 2886-2899.

Moore, M.J. 2005. From birth to death: The complex lives of eukaryotic mRNAs. Science 309(5740): 1514-1518.

Mowry, K. and Melton, D. 1992. Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in Xenopus oocytes. Science 255(5047): 991-994.

Munro, T.P., Magee, R.J., Kidd, G.J., Carson, J.H., Barbarese, E., Smith, L.M., and Smith, R. 1999. Mutational analysis of a heterogeneous nuclear ribonucleoprotein A2 response element for RNA trafficking. Journal of Biological Chemistry 274(48): 34389-34395.

Muslimov, I.A., Titmus, M., Koenig, E., and Tiedge, H. 2002. Transport of Neuronal BC1 RNA in Mauthner Axons. The Journal of Neuroscience 22(11): 4293-4301.

Nairn, A.C., Matsushita, M., Nastiuk, K., Horiuchi, A., Mitsui, K., Shimizu, Y., and Palfrey, H.C. 2001. Elongation factor-2 phosphorylation and the regulation of protein synthesis by calcium. Prog Mol Subcell Biol 27: 91-129.

Nakielny, S. and Dreyfuss, G. 1996. The hnRNP C proteins contain a nuclear retention sequence that can override nuclear export signals. Journal of Cell Biology 134(6): 1365-1373.

Neugebauer, K.M. 2002. On the importance of being co-transcriptional. Journal of Cell Science 115(20): 3865-3871.

Nguyen Chi, M., Chalmel, F.d.r., Agius, E., Vanzo, N., Khabar, K.S.A., JÃ©gou, B., and Morello, D. 2009. Temporally Regulated Traffic of HuR and Its Associated ARE-Containing mRNAs from the Chromatoid Body to Polysomes during Mouse Spermatogenesis. PLoS ONE 4(3): e4900.

Norvell, A., Kelley, R.L., Wehr, K., and Schupbach, T. 1999. Specific isoforms of Squid, a Drosophila hnRNP, perform distinct roles in Gurken localization during oogenesis. Genes & Development 13(7): 864-876.

Ostroff, L.E., Fiala, J.C., Allwardt, B., and Harris, K.M. 2002. Polyribosomes Redistribute from Dendritic Shafts into Spines with Enlarged Synapses during LTP in Developing Rat Hippocampal Slices. Neuron 35(3): 535-545.

Page, J., Suja, J.A., Santos, J.L., and Rufas, J.S. 1998. Squash Procedure for Protein Immunolocalization in Meiotic Cells. Chromosome Research 6(8): 639-642.

Pan, F., Huttelmaier, S., Singer, R.H., and Gu, W. 2007. ZBP2 facilitates binding of ZBP1 to beta-actin mRNA during transcription. Mol Cell Biol 27(23): 8340-8351.

Paquin, N. and Chartrand, P. 2008. Local regulation of mRNA translation: new insights from the bud. Trends in Cell Biology 18(3): 105-111.

Parvinen, M. and Parvinen, L.M. 1979. Active Movements of the Chromatoid Body - Possible Transport Mechanism for Haploid Gene-Products. Journal of Cell Biology 80(3): 621-628.

Percipalle, P. 2009. The long journey of actin and actin-associated proteins from genes to polysomes. Cellular and Molecular Life Sciences 66(13): 2151-2165.

Percipalle, P., Jonsson, A., Nashchekin, D., Karlsson, C., Bergman, T., Guialis, A., and Daneholt, B. 2002. Nuclear actin is associated with a specific subset of hnRNP A/B-type proteins. Nucleic Acids Research 30(8): 1725-1734.

Pickering, B.M. and Willis, A.E. 2005. The implications of structured 5 ' untranslated regions on translation and disease. Seminars in Cell & Developmental Biology 16(1): 39-47.

Pinol-Roma, S. and Dreyfuss, G. 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature 355(6362): 730-732.

Raju, C.S., Fukuda, N., Lopez-Iglesias, C., Goritz, C., Visa, N., and Percipalle, P. In neurons, activity-dependent association of dendritically transported mRNA transcripts with the transacting factor CBF-A is mediated by A2RE/RTS elements. Mol Biol Cell: mbc.E10-11-0904.

Raju, C.S., Goritz, C., Nord, Y., Hermanson, O., Lopez-Iglesias, C., Visa, N., Castelo-Branco, G., and Percipalle, P. 2008. In Cultured Oligodendrocytes the A/B-type hnRNP CBF-A Accompanies MBP mRNA Bound to mRNA Trafficking Sequences. Mol Biol Cell 19(7): 3008-3019.

Reck-Peterson, S.L., Tyska, M.J., Novick, P.J., and Mooseker, M.S. 2001. The Yeast Class V Myosins, Myo2p and Myo4p, Are Nonprocessive Actin-Based Motors. The Journal of Cell Biology 153(5): 1121-1126.

Rehbein, M., Kindler, S., Horke, S., and Richter, D. 2000. Two trans-acting rat-brain proteins, MARTA1 and MARTA2, interact specifically with the dendritic targeting element in MAP2 mRNAs. Molecular Brain Research 79(1-2): 192-201.

Rigo, F. and Martinson, H.G. 2008. Functional Coupling of Last-Intron Splicing and 3'-End Processing to Transcription In Vitro: the Poly(A) Signal Couples to Splicing before Committing to Cleavage. Mol Cell Biol 28(2): 849-862.

Rook, M.S., Lu, M., and Kosik, K.S. 2000. CaMKIIalpha 3' untranslated region-directed mRNA translocation in living neurons: visualization by GFP linkage. J Neurosci 20(17): 6385-6393.

Ross, A.F., Oleynikov, Y., Kislauskis, E.H., Taneja, K.L., and Singer, R.H. 1997. Characterization of a beta-actin mRNA zipcode-binding protein. Molecular and Cellular Biology 17(4): 2158-2165.

Sánchez-Carbente, M.d.R., DesGroseillers, L., Wayne S. Sossin, J.-C.L.V.F.C., and Sylvie, B. 2008. Chapter 3 Understanding the importance of mRNA transport in memory. In Progress in Brain Research, Vol Volume 169, pp. 41-58. Elsevier.

Sassone-Corsi, P. 2002. Unique chromatin remodeling and transcriptional regulation in spermatogenesis. Science 296(5576): 2176-2178.

Saunders, P.T.K., Millar, M.R., Maguire, S.M., and Sharpe, R.M. 1992. Stage-Specific expression of rat transition protein 2 mrna and possible localization to the chromatoid body of step 7 spermatids by in situ hybridization using a nonradioactive riboprobe. Molecular Reproduction and Development 33(4): 385-391.

Scheetz, A.J., Nairn, A.C., and Constantine-Paton, M. 2000. NMDA receptor-mediated control of protein synthesis at developing synapses. Nature Neuroscience 3(3): 211-216.

Schratt, G.M., Tuebing, F., Nigh, E.A., Kane, C.G., Sabatini, M.E., Kiebler, M., and Greenberg, M.E. 2006. A brain-specific microRNA regulates dendritic spine development (vol 439, pg 283, 2006). Nature 441(7095): 902-902.

Schuldt, A.J., Adams, J.H.J., Davidson, C.M., Micklem, D.R., Haseloff, J., St Johnston, D., and Brand, A.H. 1998. Miranda mediates asymmetric protein and RNA localization in the developing nervous system. Genes & Development 12(12): 1847-1857.

Shan, J.G., Munro, T.P., Barbarese, E., Carson, J.H., and Smith, R. 2003. A molecular mechanism for mRNA trafficking in neuronal dendrites. Journal of Neuroscience 23(26): 8859-8866.

Shang, P., Baarends, W.M., Hoogerbrugge, J., Ooms, M.P., van Cappellen, W.A., de Jong, A.A.W., Dohle, G.R., van Eenennaam, H., Gossen, J.A., and Grootegoed, J.A.

Functional transformation of the chromatoid body in mouse spermatids requires testis-specific serine/threonine kinases. J Cell Sci 123(3): 331-339.

Shepard, K.A., Gerber, A.P., Jambhekar, A., Takizawa, P.A., Brown, P.O., Herschlag, D., DeRisi, J.L., and Vale, R.D. 2003. Widespread cytoplasmic mRNA transport in yeast: Identification of 22 bud-localized transcripts using DNA microarray analysis. Proceedings of the National Academy of Sciences of the United States of America 100(20): 11429-11434.

Shiina, N., Shinkura, K., and Tokunaga, M. 2005. A novel RNA-binding protein in neuronal RNA granules: regulatory machinery for local translation. J Neurosci 25(17): 4420-4434.

Shiota, C., Miura, M., and Mikoshiba, K. 1989. Developmental profile and differential localization of mRNAs of myelin proteins (MBP and PLP) in oligodendrocytes in the brain and in culture. Brain Res Dev Brain Res 45(1): 83-94.

Shyu, A.B. and Wilkinson, M.F. 2000. The double lives of shuttling mRNA binding proteins. Cell 102(2): 135-138.

Sierakowska, H., Szer, W., Furdon, P.J., and Kole, R. 1986. Antibodies to hnRNP core proteins inhibit in vitro splicing of human Î²-globin pre-mRNA. Nucleic Acids Research 14(13): 5241-5254.

Singer, R.H. 2008. Highways for mRNA Transport. Cell 134(5): 722-723.

Smith, R. 2004. Moving molecules: mRNA trafficking in Mammalian oligodendrocytes and neurons. Neuroscientist 10(6): 495-500.

St Johnston, D. 2005. Moving messages: the intracellular localization of mRNAs. Nat Rev Mol Cell Biol 6(5): 363-375.

St Johnston, D., Beuchle, D., and Nusslein-Volhard, C. 1991. Staufen, a gene required to localize maternal RNAs in the Drosophila egg. Cell 66(1): 51-63.

Steward, O. and Levy, W.B. 1982. Preferential Localization of Polyribosomes under the Base of Dendritic Spines in Granule Cells of the Dentate Gyrus. Journal of Neuroscience 2(3): 284-291.

Steward, O. and Worley, P.F. 2001. Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation. Neuron 30(1): 227-240.

Stjohnston, D., Brown, N.H., Gall, J.G., and Jantsch, M. 1992. A Conserved Double-Stranded Rna-Binding Domain. Proceedings of the National Academy of Sciences of the United States of America 89(22): 10979-10983.

Sung, Y.J., Weiler, I.J., Greenough, W.T., and Denman, R.B. 2004. Selectively enriched mRNAs in rat synaptoneurosomes. Molecular Brain Research 126(1): 81-87.

Svitkin, Y.V., Ovchinnikov, L.P., Dreyfuss, G., and Sonenberg, N. 1996. General RNA binding proteins render translation cap dependent. EMBO J 15(24): 7147-7155.

Söderström, K.-O. 1981. Labelling of the chromatoid body by [3H]uridine in rat pachytene spermatocytes. Experimental Cell Research 131(2): 488-491.

Söderström, K.-O. and Parvinen, M. RNA synthesis in different stages of rat seminiferous epithelial cycle. Molecular and Cellular Endocrinology 5(3-4): 181-199.

Takizawa, P.A., Sil, A., Swedlow, J.R., Herskowitz, I., and Vale, R.D. 1997. Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. Nature 389(6646): 90-93.

Tanaka, H. and Baba, T. 2005. Gene expression in spermiogenesis. Cellular and Molecular Life Sciences 62(3): 344-354.

Tang, S.J., Meulemans, D., Vazquez, L., Colaco, N., and Schuman, E. 2001. A role for a rat homolog of staufen in the transport of RNA to neuronal dendrites. Neuron 32(3): 463-475.

Tang, S.J., Reis, G., Kang, H., Gingras, A.C., Sonenberg, N., and Schuman, E.M. 2002. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. Proc Natl Acad Sci U S A 99(1): 467-472.

Taniguchi, I. and Ohno, M. 2008. ATP-Dependent Recruitment of Export Factor Aly/REF onto Intronless mRNAs by RNA Helicase UAP56. Mol Cell Biol 28(2): 601-608.

Tansey, F.A., Zhang, H., and Cammer, W. 1996. Expression of carbonic anhydrase II mRNA and protein in oligodendrocytes during toxic demyelination in the young adult mouse. Neurochem Res 21(4): 411-416.

Tian, Q.B., Nakayama, K., Okano, A., and Suzuki, T. 1999. Identification of mRNAs localizing in the postsynaptic region. Molecular Brain Research 72(2): 147-157.

Tiruchinapalli, D.M., Oleynikov, Y., Kelic, S., Shenoy, S.M., Hartley, A., Stanton, P.K., Singer, R.H., and Bassell, G.J. 2003. Activity-dependent trafficking and dynamic localization of zipcode binding protein 1 and beta-actin mRNA in dendrites and spines of hippocampal neurons. Journal of Neuroscience 23(8): 3251-3261.

Tongiorgi, E., Righi, M., and Cattaneo, A. 1997. Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. Journal of Neuroscience 17(24): 9492-9505.

Toyooka, Y., Tsunekawa, N., Takahashi, Y., Matsui, Y., Satoh, M., and Noce, T. 2000. Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. Mechanisms of Development 93(1-2): 139-149.

Trcek, T. and Singer, R.H. The cytoplasmic fate of an mRNP is determined cotranscriptionally: exception or rule? Genes & Development 24(17): 1827-1831.

Tsai-Morris, C.-H., Sheng, Y., Lee, E., Lei, K.-J., and Dufau, M.L. 2004. Gonadotropin-regulated testicular RNA helicase (GRTH/Ddx25) is essential for spermatid development and completion of spermatogenesis. Proceedings of the National Academy of Sciences of the United States of America 101(17): 6373-6378.

Unhavaithaya, Y., Hao, Y., Beyret, E., Yin, H., Kuramochi-Miyagawa, S., Nakano, T., and Lin, H. 2009. MILI, a PIWI-interacting RNA-binding Protein, Is Required for Germ Line Stem Cell Self-renewal and Appears to Positively Regulate Translation. Journal of Biological Chemistry 284(10): 6507-6519.

Weil, T.T., Forrest, K.M., and Gavis, E.R. 2006. Localization of bicoid mRNA in Late Oocytes Is Maintained by Continual Active Transport. Developmental cell 11(2): 251-262.

Weil, T.T., Parton, R., Davis, I., and Gavis, E.R. 2008. Changes in bicoid mRNA Anchoring Highlight Conserved Mechanisms during the Oocyte-to-Embryo Transition. Current Biology 18(14): 1055-1061.

Wells, D.G. 2006. RNA-binding proteins: a lesson in repression. J Neurosci 26(27): 7135-7138.

Verity, A.N. and Campagnoni, A.T. 1988. Regional expression of myelin protein genes in the developing mouse brain: In situ hybridization studies. Journal of Neuroscience Research 21(2-4): 238-248.

White, R., Gonsior, C., KrÃ¤mer-Albers, E.-M., StÃ¶hr, N., HÃ¼ttelmaier, S., and Trotter, J. 2008. Activation of oligodendroglial Fyn kinase enhances translation of mRNAs transported in hnRNP A2â€“dependent RNA granules. The Journal of Cell Biology 181(4): 579-586.

Visa, N., AlzhanovaEricsson, A.T., Sun, X., Kiseleva, E., Bjorkroth, B., Wurtz, T., and Daneholt, B. 1996. A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. Cell 84(2): 253-264.

Visa, N., Alzhanova-Ericsson, A.T., Sun, X., Kiseleva, E., Björkroth, B., Wurtz, T., and Daneholt, B. 1996. A Pre-mRNA-Binding Protein Accompanies the RNA from the Gene through the Nuclear Pores and into Polysomes. Cell 84(2): 253-264.

Wu, L., Wells, D., Tay, J., Mendis, D., Abbott, M.A., Barnitt, A., Quinlan, E., Heynen, A., Fallon, J.R., and Richter, J.D. 1998. CPEB-mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of alpha-CaMKII mRNA at synapses. Neuron 21(5): 1129-1139.

Yisraeli, J.K., Sokol, S., and Melton, D.A. 1990. A two-step model for the localization of maternal mRNA in Xenopus oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. Development 108(2): 289-298.

Zaidi, S.H. and Malter, J.S. 1995. Nucleolin and heterogeneous nuclear ribonucleoprotein C proteins specifically interact with the 3'-untranslated region of amyloid protein precursor mRNA. The Journal of biological chemistry 270(29): 17292-17298.

Zhang, H.L., Eom, T., Oleynikov, Y., Shenoy, S.M., Liebelt, D.A., Dictenberg, J.B., Singer, R.H., and Bassell, G.J. 2001. Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. Neuron 31(2): 261