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Candidate RNA-Binding Proteins Regulating Extrasomatic mRNA Targeting and Translation in Mammalian neurons

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Abstract

In mammalian neurons, long-lasting changes in the efficacy of individual synapses depend on the synthesis of new proteins. To maintain specificity, neuronal cells have to ensure that these newly synthesized proteins accumulate at the appropriate subpopulation of synapses. One way that neurons have solved this challenge appears to be the local translation of extrasomatic mRNAs in dendrites and at postsynaptic sites. Mechanisms, which regulate the targeting, translation, and stability of dendritic mRNAs, involve an organized interaction between *cis*-acting elements of localized transcripts and *trans*-acting RNA-binding proteins. The molecular identity and cellular functions of *trans*-acting factors that are likely to play an important role in post-transcriptional processing of extrasomatic transcripts in mammalian neurons are now being elucidated.

Index Entries: Dendritic RNA sorting; extrasomatic translation; ribonucleoprotein particle; *trans*-acting factor; synaptic activity.

Introduction

Neurons in the central nervous system (CNS) of mammals possess distinct cellular compartments that are highly diverse with respect to their protein repertoires. In particular, synapses serving as communication sites between nerve cells are equipped with a highly specialized set of molecules. Via its synaptic contacts, an individual neuron may receive input signals from thousands of different cells. A synapse that is stimulated by a given axon often establishes a "tag" that distinguishes the activated contact site from the many others, which remained

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inactive *(1).* This tag enables synapses to establish a history of stimulatory events. Synaptic response to activation is influenced by the stimulation history of a particular synapse, a phenomenon that underlies learning and memory and is referred to as "synaptic plasticity" *(2,3).* Synaptic plasticity involves a synapse-specific modification of the protein composition. This adaptation is achieved by at least two cellular mechanisms, namely, selective targeting of somatically synthesized proteins to synapses and a regulated translation of dendritically localized mRNAs near synaptic contacts *(4–8).* The significance of the latter mechanism was supported by the identification of polysomes in dendritic shafts *(9–10)* and the detection of protein synthesis in isolated dendrites *(11–14).* In mammalian neurons, dendritic RNA sorting is an energy-dependent mechanism that requires cytoskeletal filaments *(4,6,15).* Selected mRNAs found in dendrites include transcripts encoding the alpha subunit of the Ca^{2+}/calmodulin-dependent protein kinase II (α-CaMKII) *(16),* the somatodendritic microtubule-associated protein 2 (MAP2) *(17–19);* the product of an activity-regulated gene (arg3.1/Arc) *(20,21);* a dendritic protein of unknown function, called dendrin *(22);* precursors of the neuropeptides vasopressin and oxytocin *(23);* and ligatin *(24).* An ordered interaction between *cis*-acting elements of these transcripts and *trans*-acting proteins appears to regulate cytoplasmic mRNA targeting and site-specific translation *(25).* Recently, *cis*-acting elements involved in dendritic mRNA targeting or translation were characterized in transcripts encoding MAP2 *(26),* α-CaMKII *(27–29),* vasopressin *(30),* and ligatin *(24).* In this review, we summarize recent data on a number of RNA-binding proteins that are likely to represent *trans*-acting proteins involved in post-transcriptional processing of extrasomatic transcripts in mammalian neurons. These *trans*-factors are thought to direct multiple steps of mRNA metabolism, such as nuclear export, directed cytoplasmic trafficking along cytoskeletal filaments, translational silencing of mRNAs en route, stabilization of moving or dendritically anchored transcripts, and initiation of mRNA translation upon specific synaptic stimulation.

Staufen, an Evolutionary Conserved Cytoplasmic RNA-Targeting Protein

The double-stranded (ds) RNA-binding protein Staufen was first identified in *Drosophila* as product of a maternal effect gene. In embryos, it is involved in the correct formation of the anteroposterior body pattern *(31,32),* This effect is due to the protein's role in localizing maternal *bicoid*- and *oskar*-transcripts to the opposite poles of oocytes *(33)* and the translational derepression of *oskar*-transcripts at the posterior pole *(34).* Staufen was also shown to be involved in the proper targeting of *prospero*-mRNA in dividing neuroblasts, the neural precursor cells in *Drosophila (35–37).* Staufen contains five areas with significant similarity to dsRNA-binding domains (dsRBDs) of the *Xenopus laevis* protein Xlrbpa *(38).* dsRBD1, 3, and 4 independently interact with dsRNA in vitro *(34,38).* dsRBD2 and 5 match only the carboxy-terminal part of the dsRBD consensus motif and are not able to bind dsRNA in vitro. dsRBD3 is required for Staufen-dependent localization of *bicoid* and *oskar* mRNAs in vivo *(39).* NMR-studies revealed that dsRBD3 folds into a compact αββββα configuration, which interacts optimally with RNA stem-loop structures containing 12 uninterrupted base pairs *(39).* dsRBD2 is essential for microtubule-dependent localization of oskar transcripts in oocytes *(34).* dsRBD5 possesses a dual function for the translational derepression of posterior *oskar* mRNAs in oocytes *(34)* and the actin-dependent localization of *prospero* mRNA in neuroblasts *(36,40).* Thus, distinct domains of *Drosophila* Staufen mediate in vivo RNA-binding, translational control, and microtubule- and microfilament-based mRNA sorting.

Recently, mammalian Staufen orthologues were identified in mouse, rat, and human *(41–44).* Mammalian Staufen isoforms are present in many tissues, including brain. They are

devoid of the amino-terminal part of *Drosophila* Staufen and thus lack dsRBD1. Identities between mammalian and fly proteins are highest in the dsRBDs and range between 47 and 66%. In human, four alternatively spliced mRNAs give rise to two different 54- and 63-kDa isoforms, which possess distinct amino-terminal regions *(43).* In rat and mouse, the Stau+I6/Stau^{i} isoform carries a six amino acid residue stretch within dsRBD3 that is absent from the shorter Stau-I6 isoform *(44,45).* This six amino acid residue region leads to a drastically reduced in vitro and in vivo dsRNA-binding ability of Stau+I6/Stau^{i} as compared to Stau-I6 *(44,45).* Similar to *Drosophila* Staufen, the mammalian orthologues bind dsRNA in a sequence-independent manner both in vitro and in the yeast tri-hybrid system *(42–44).* However, specific in vivo RNA-targets still need to be identified.

In rat hippocampal neurons, endogenous and recombinant Staufen is somatodendritically localized and forms macromolecular complexes along dendrites that appear to be associated with microtubules *(41,44,46).* In dendrites, recombinant Staufen particles show a saltatory, bi-directional, and microtubule-based movement and contain RNA as identified with the nucleic acid dye SYTO14 *(46).* Interestingly, similar RNA-containing granules in dendritic shafts were also shown to comprise ribosomal components, elongation factor 1α, and poly(A)-RNA *(47).* Although immunoelectron microscopy did not reveal a colocalization of Staufen and ribosomes along dendritic shafts *(41),* the protein was found to cosediment with polysomes prepared from non-neuronal HeLa cells *(42).* Taken together, these data imply that neuronal Staufen associates with mRNA targeting complexes that move along dendritic microtubules. Currently, it is not known whether Staufen-containing RNP complexes *en route* are translationally active or silent. A regulated expression of distinct Staufen isoforms may be used to adjust the total amount of RNA and select mRNA target species associated with Staufen mRNP particles *(44,45).* This model raises the question of which proteins and mRNAs are specifically associated with dendritically localized Staufen granules in vivo. In this context, it is interesting to note that although mammalian homologs of *bicoid* and *oskar* were not identified, a *prospero* homolog, *prox1,* is found in the mouse nervous system during development *(48).* Whether prox1 mRNA is sorted into dendrites awaits further analysis.

Disease-Linked Fragile X Mental Retardation Protein 1

Fragile X mental retardation protein (FMRP) is an RNA-binding protein that is encoded by the fragile X mental retardation-1 (FMR1) gene *(49).* Massive expansion of CGG triplet repeats in the FMR1 gene leads to the fragile X syndrome, a common form of inherited mental retardation *(49).* In fragile X patients, expanded triplet repeats are hypermethylated and FMR1 gene expression is repressed, which results in the absence of FMRP and subsequent mental retardation *(49).*

In mammals, the FMR1 gene is expressed in many tissues including brain *(50).* FMRP contains two types of RNA-binding motifs, two ribonucleoprotein K homology domains (KH domains) and a cluster of arginine and glycine residues (RGG box) *(51,52).* A patient carrying an isoleucine to asparagine amino acid residue exchange at position 304 (I304N), which is located at the second KH domain, exhibits unusually severe mental retardation *(53).* Two FMRP homologs, FXR1P and FXR2P, were identified *(54,55).* In cells from fragile X patients and FMR-1 knockout mice, FXR1P and FXR2P levels are unchanged *(56).* Thus, despite their structural similarity with FMRP both proteins do not appear to compensate for FMRP functions.

In vitro, FMRP preferentially interacts with poly(G) and poly(U) instead of poly(C) and poly(A) and it only binds to selective brain transcripts, including the 3′ untranslated regions (3′-UTRs) of myelin basic protein (MBP) and the FMR1 mRNA *(57).* However,

transcripts that interact with FMRP in vivo still need to be determined. Deletion analysis revealed that only the first KH domain but not the second associates with RNA *(58).* This is consistent with the finding that FMRP carrying the I304N point mutation in the second KH domain binds RNA *(57).* The first KH domain preferentially interacts with poly(G). In addition, both amino- and carboxy-terminal parts of FMRP interact with RNA. Similar to the first KH domain alone, the entire amino-terminal region favouritely binds to poly(G). In contrast, the carboxy-terminus containing the RGG box shows sequence-independent RNA-binding *(58).* Thus, FMRP contains multiple RNA interaction sites and sequence specificity may be achieved by a coordinated interplay between different elements.

In cells, FMRP associates with actively translating polyribosomes in an RNA-dependent mode via messenger ribonucleoprotein (mRNP) particles *(59,60).* Upon the dissociation of ribosomes into subunits, FMRP is released into ~660 kDa complexes, a size typical for large mRNP particles *(61,62).* Although the I304N mutant isoform is normally expressed and associated with cytoplasmic RNAs in cells, it associates with smaller-sized mRNP particles and does not couple to actively translating polyribosomes *(62).* The severe phenotype of patients carrying the I304N mutation implies that an association of FMRP with polyribosomes is functionally significant. Whereas FMRP can form dimers, the I304N mutant isoform fails to homomultimerize *(63).* Thus, dimerization seems to be critical for polyribosome association. Some of the molecular components of FMRP-containing mRNPs were recently identified. Along with FMRP, at least six additional proteins form the mRNP particle, including FXR1P and FXR2P as well as nucleolin, a known component of other mRNPs *(64,65).* Other associated proteins remain to be identified. Its polyribosome association hints towards a function of FMRP in regulating mRNA translation and stability. This assumption is supported by recent data showing that FMRP can suppress translation both in rabbit reticulocyte lysates and microinjected *Xenopus laevis* oocytes *(63,66).* Interestingly, the I304N mutation disrupts this translational blockade.

In neurons, FMRP predominantly resides in the somatodendritic cytoplasm *(67).* In neuronal perikarya, it accumulates in areas rich in free ribosomes, especially near or between cisternae of the rough endoplasmic reticulum. In dendritic shafts, FMRP is concentrated in the proximity of cisternae of the smooth endoplasmic reticulum, at dendritic branch points, and at the origins of dendritic spine necks. All of these are known regions of polyribosome accumulation. In spine heads, it is either free in the cytoplasm or associated with the spine apparatus. Moreover, FMRP co-fractionates with synaptosomal ribosomes *(67)* and stimulation of metabotropic glutamate receptors increases FMRP translation in rat synaptoneurosomes *(68).* Thus, FMRP may play a role in the synaptic-activity-dependent dendritic translation of its own mRNA as well as the local synthesis of other proteins at postsynaptic sites.

Despite its predominant cytoplasmic localization, FMRP contains both functional nuclear localization (NLS) and nuclear export signals (NES) *(61)* suggesting that FMRP shuttles between the nucleus and cytoplasm. Using immunogold electron microscopy, FMRP was indeed detected in neuronal nuclei and nuclear pores *(67).* FMRP can be phosphorylated by Fes nonreceptor tyrosine kinase and the tyrosine-phosphorylated protein is primarily located in the nucleus *(69).* Thus, nuclear-cytoplasmic shuttling of FMRP may be regulated via its phosphorylation status. Similar to FMRP, FXR1P, and FXR2P also contain NLS and NES motifs *(55).* However, within the nucleus FMRP resides within the nucleoplasm, whereas FXR2P is found at nucleoli *(70).* Moreover, a novel nuclear RNA-binding protein, NUFIP, interacts with FMRP, but does not bind to FXR1P and FXR2P *(64).* In summary, the abovementioned data imply that nascent FMRP, FXR1P, and FXR2P enter the nucleus and assemble into distinct mRNP complexes that are subsequently targeted to the cytoplasm. In the cytoplasm, all three proteins appear to form a common mRNP particle.

Thus, FMRP and its two homologs may play a role in nuclear mRNA export, cytoplasmic transcript targeting, and the regulation of mRNA stability and translation at synapses. Consistent with this hypothesis, brain neurons of fragile X patients and FMR1 knockout mice possess abnormal dendritic spines *(71,72).* In fragile X patients, the absence of FMRP may result in a misregulated protein synthesis during synapse development, thus causing mental retardation. In patients carrying the I304N point mutation, FMRP does not dimerize and mRNP particles containing FMRP fail to associate with polyribosomes, thereby sequestering FMRP-bound transcripts from translation. Interestingly, in FMR-1 knockout mice the dendritic mRNA localization patterns of MAP2, αCaMKII, dendrin, and arc/arg3.1 mRNAs are unchanged, excluding FMRP as an obligatory component of the extrasomatic mRNA targeting machinery in neurons *(73).* However, it remains possible that FMRP plays an essential role in dendritic targeting of some other mRNAs and that FXR1P and FXR2P adopt transcript localization functions of FMRP in FMR-1 knockout mice.

Function of Embryonic Lethal Abnormal Vision-Like RNA-Binding Proteins in GAP-43 and tau mRNA Targeting in Neurons

In *Drosophila,* the embryonic lethal abnormal vision (ELAV) protein is involved in neuronal differentiation and maintenance *(74,75).* Vertebrate orthologues were described in human, rat, mouse, chicken, and *Xenopus laevis (76–78).* Humans possess four ELAV-like proteins, HuB (including isoforms Hel-N1 and Hel-N2), HuC, HuD, and HuR. HuR is ubiquitously expressed, whereas the other family members are neuron- and gonad-specific. All family members contain three RNA recognition motifs (RRMs) consisting of 90–100 amino acid residues with a three-dimensional βαββαβ structure *(79).* The first two RRMs appear in tandem and are separated from the third motif by a stretch of basic amino acids. In HuD, the first two RRMs are required for its specific binding to a conserved regulatory element in the 3′UTR of the GAP-43 mRNA *(80).* In Hel-N1, the third RRM is essential for its interaction with RNA *(81).* ELAV-like proteins associate in vitro with a broad range of poly(A) RNAs, which encode cellular-growth regulatory proteins and contain AU-rich elements (AREs) in their 3′-UTRs *(76–78).* Binding of ELAV-like proteins to AREs confers prolonged RNA stability and enhances target mRNA translation. In neuronal somata and dendrites, ELAV-like proteins reside in microtubule-bound mRNP granules (α-complexes) *(82).* Microtubule association of α-complexes appears to be necessary for the formation of larger polysome-containing β-complexes. The observation that HuR stabilizes ARE-containing mRNAs and undergoes nucleo-cytoplasmic shuttling *(83,84)* indicates that HuR binds target mRNAs in the nucleus and remains transcript-associated upon nuclear export and cytoplasmic localization, thereby providing ongoing protection from RNA degradation. Furthermore, HuR interacts with three different shuttling proteins (SETα, SETβ, and pp32), which were previously characterized as inhibitors of protein phosphatase 2A *(77).* In summary, these findings are consistent with a potential role of ELAV-like proteins and protein phosphatase 2A in nucleo-cytoplasmic and extrasomatic mRNA targeting, translation, and stabilization. Among neuronal transcripts binding to ELAV-like proteins are GAP-43 *(80)* and tau mRNAs *(85),* that are targeted to dendrites *(86)* and axon hillocks *(87),* respectively. The neuron-specific phosphoprotein GAP-43 is involved in the regeneration and remodeling of neuronal connections *(88).* HuD specifically interacts with 26 nucleotides of the GAP-43 3′-UTR *(80).* The functional consequences of this interaction are unknown. In neurons, the microtubule-associated protein tau is sorted to axons and appears to regulate the stability of axonal microtubules and neuronal polarity. In PC12 cells, HuD binds to a U-rich region of the tau 3′-UTR *(85).* Treatment of PC12 cells with antisense oligonucleotides directed against HuD blocks the induction of neurite

outgrowth and decreases tau mRNA levels. Moreover, Hel-N1 overexpression in hNT2 cells induces spontaneous neurite formation and mRNA recruitment to active polysomes *(89).* Taken together, the current data suggest that selected neuronal mRNAs bind ELAV-like proteins to form microtubule-associated mRNP granules, which, in turn, associate with polysomes to form translational complexes. In these mRNP particles, ELAV-like proteins may direct post-transcriptional gene expression by regulating cytoplasmic mRNA localization, translation, and stability. It is important to note that in vitro ELAV-like proteins bind AREs in a large number of transcripts, which are localized to distinct cellular subregions, as described earlier for GAP-43 and tau transcripts. Therefore, cytoplasmic sorting and extrasomatic translation of these mRNAs in neurons is likely to involve additional "specificity factors," which direct target mRNA recognition in vivo.

Calcium-Regulated RNA-Binding of Visinin-Like Protein

In vertebrates, the visinin-like protein VILIP is a neural RNA-binding protein present in neuronal perikarya, dendrites, and some axons in several brain regions, including mammalian hippocampus *(90–93).* It comprises four canonical calcium-binding EF-hands and seems to be involved in calcium-signaling events, such as a ligand-activated cAMP formation *(94,95).* Although a single full-length dsRBD in rat brain VILIP possesses only 11% similarity to other dsRBDs, as compared to an average 28.6% similarity among all other analyzed full-length dsRBDs, amino acid residues in important key positions of dsRBDs are conserved in VILIP *(38,96).* Rat VILIP specifically binds dsRNA in vitro in a calcium-dependent manner *(96).* Interestingly, two EF-hands overlap the dsRBD, suggesting that calcium binding leads to a conformational change enabling the protein to bind dsRNA. Mobility shift assays revealed that VILIP forms a large RNA-protein complex with 200 bp dsRNAs and that it binds far less efficiently to shorter dsRNAs. VILIP specifically interacts in vitro with the 3′-UTR but not the coding region of the neurotrophin receptor trkB mRNA. In primary hippocampal neurons, trkB transcripts are sorted to dendrites in an activity-dependent manner *(97).* These observations suggest a model, in which neuronal activity increases postsynaptic, intracellular calcium concentrations, thereby stimulating a calcium-dependent association and dendritic translocation of VILIP and trkB transcripts. Although VILIP colocalizes with actin filaments in rat PC12 cells *(98),* it remains to be shown whether dendritic sorting of VILIP-containing RNP complexes in neurons involves cytoskeletal filaments *(96).*

Zipcode Binding Protein-1 and β-actin mRNA Sorting to Neuronal Growth Cones

Cytoplasmic sorting of β-actin transcripts to the leading edge of chicken fibroblasts depends on a 54-nucleotide *cis*-acting targeting element in the 3′-UTR, referred to as zipcode *(99).* The zipcode binding protein-1 (ZBP-1) containing two RRMs and four KH domains interacts with the 5′-half of the zipcode *(100).* Different mutations within this half reduce both cytoplasmatic localization capacity and binding to ZBP-1. Thus, ZBP-1 has been suggested to regulate β-actin mRNA sorting in fibroblasts. ZBP-1 is a chicken homolog of the human IGF-II mRNA-binding protein (IMP) familiy *(101).* The three human IMPs bind to the 5′UTR of the insulin-like growth factor mRNA, thus potentially regulating its translation. Notably, Vg1-RBP/Vera, a *trans*-factor binding the *cis*-acting vegetal localization element of Vg1 transcripts in *Xenopus laevis* oocytes, is a frog orthologue of ZBP-1 *(102,103).* Since β-actin and Vg1 mRNA sorting depends on microfilaments and microtubules *(100,103),* respectively, this *trans*-factor may mediate cytoplasmic mRNA localization

along different cytoskeletal filaments. Recombinant mouse CRD-BP, another member of the ZBP-1 family of RNA-binding proteins, specifically interacts in vitro with the *cis*-acting coding region determinant of c-myc transcripts, a sequence element involved in regulating c-myc mRNA stability *(104,105).* In addition, ZBP-1 related proteins contain nuclear import and export signals and may therefore regulate nuclear export and cytoplasmic fate of specific mRNAs in different cell systems. In developing primary cerebrocortical neurons, β-actin transcripts form granules situated in neurites and growth cones, which colocalize with translational components *(106).* In addition, neurotrophin-3 induces a cAMP-dependent mechanism that promotes microtubule-based sorting of β-actin mRNAs into growth cones *(107).* Although these findings are compatible with the hypothesis that ZBP-1 is involved in extrasomatic β-actin mRNA sorting in neurons, functional confirmation of such a role is still lacking.

Translin-Mediated Post-transcriptional Regulation of α-CaMKII Expression

Translin, also referred to as testis brain RNA-binding protein, was identified as a protein interacting with single-stranded DNA *(108).* In vitro studies showed that it also binds to conserved Y and H sequence elements present in many brain and testis mRNAs *(109–111).* A carboxy-terminal leucine zipper in Translin is important for both DNA- and RNA-binding *(112,113).* Whereas RNA-binding requires two additional basic domains, only the second of these domains is needed for an interaction with DNA *(114).* The Translin-associated factor X (Trax) modulates the RNA- and DNA-binding activity of Translin in vitro *(114).* A Translin/Trax heterodimer does not bind to RNA, but it shows enhanced binding to specific single-stranded DNA sequences. Trax alone does neither bind to RNA nor DNA in vitro *(114).* Finally, Translin and Trax were co-immunoprecipitated from cytosolic brain and testis extracts, indicating that the in vivo RNA- and DNA-binding capacity of Translin is regulated via its interaction with Trax *(115).* In addition, Translin interacts with microtubules both in vitro and in vivo *(116,117).* Thus, it may function as an anchoring protein that docks RNA onto microtubules.

In rat brain, Translin and Trax are expressed in neurons of different brain regions *(115).* Subcellular fractionation revealed that both proteins are highly enriched in the cytosolic fraction compared with the nuclear fraction, consistent with a role in RNA, rather than DNA, processing. In cerebellar Purkinje cells and hippocampal and neocortical pyramidal neurons in adult rat brain, Translin exhibits a somatodendritic localization pattern *(115).* Similarly, in primary cultures of developing cortical neurons, Translin was found in somata and dendrites *(118).* In contrast, a third study reports a primarily nuclear localization of Translin in adult mouse brain neurons *(119).* Whether these distinct immunostaining patterns are due to different experimental procedures remains to be shown.

In spermatocytes, Translin is a component of an RNA-binding complex implicated in suppressing translation of different mRNAs that are transcribed during early spermatogenesis *(109–111).* Moreover, it appears to be involved in RNA transport across intercellular bridges connecting developing cells *(120).* In *Xenopus laevis* oocytes, the frog homolog X-translin associates with Trax and has been implicated in the repression of maternal mRNA translation during oogenesis and embryogenesis *(121).* In the mouse brain, translin is a component of a ribonucleoprotein particle associated with BC1, a small noncoding dendritically localized RNA of unknown function *(122,123).* In a gel-shift assay, a neuronal translin-containing complex binds to a Y element in the 3′-UTR of the dendritically localized ligatin mRNA *(24).* A similar complex appears to associate with a Y element in the coding region of dendritic αCaMKII transcripts. Antisense oligonucleotides to the Translin-binding element in αCaMKII transcripts decreased

the level of this mRNA in somata and dendrites of primary rat neurons *(24).* Thus, a blockade of the Translin/αCaMKII mRNA interaction may interfere with normal RNA processing in neurons. This idea is further supported by the coimmunoprecipitation of Translin and αCaMKII transcripts from mouse brain extracts *(117).* Taken together, the above data hint towards a function of a Translin-containing mRNP particle in the regulation of dendritic mRNA transport and translation in neurons.

Activity-Dependent Regulation of αCaMKII mRNA Translation by the Cytoplasmic Polyadenylation Element Binding Protein

During early metazoan development, translation of several dormant maternal transcripts containing relatively short poly(A) tails is initiated via mRNA polyadenylation *(124,125).* Poly(A) tail elongation is mediated by two *cis*-acting elements in the 3′-UTR of the respective messages, namely the hexanucleotide polyadenylation signal AAUAAA and the cytoplasmic polyadenylation element (CPE), which has the general structure of UUUUUAU. Both elements interact with distinct *trans*-acting factors, the cleavage and polyadenylation specific factor (CPSF) and the CPE-binding protein (CPEB), respectively. CPEB contains two RRMs and a cysteine-histidine rich region (reminiscent of a zinc-finger domain), all of which are essential for RNA-binding *(126).* Together with the poly(A) polymerase, CPEB and CPSF form the core cytoplasmic polyadenylation complex *(127).*

How is cytoplasmic polyadenylation initiated? Progesterone-stimulated maturation of *Xenopus laevis* oocytes induces a transient decrease of cAMP levels, followed by the activation of Eg2, a protein kinase of the Aurora family *(128).* Activated Eg2 phosphorylates CPEB, and the phosphoprotein recruits CPSF into an active cytoplasmic polyadenylation complex, in which CPSF guides poly(A) polymerase to the 3′-end of the mRNA. Before oocyte maturation, CPEB is also involved in translational repression of maternal mRNAs, potentially via its interaction with the 5′-cap or cap-binding proteins *(124,125).* In support of this hypothesis, the CPEB-interacting protein maskin binds eIF4E, precludes an association of eIF4E and eIF4G, thereby interfering with the correct positioning of the 40S ribosomal subunit to the 5′-end of the transcript. During maturation eIF4E partially dissociates from maskin and is free to associate with eIf4G and stimulate translation.

Recently, it has become evident that CPEB may also be involved in translational regulation of dendritically sorted mRNAs in mammalian neurons. In the rodent brain, CPEB is found in several regions including hippocampus, cerebellum, and cortex *(27).* In hippocampal neurons, CPEB resides in somata, dendrites, and synapses, and is enriched in biochemical preparations of postsynaptic densities from brain. Interestingly, dendritically targeted αCaMKII mRNAs contain two CPEs *(27).* αCaMKII is essential for long-term potentiation and memory formation *(129,130).* In αCaMKII transcripts, both CPEs are situated in the 3′-UTR shortly upstream of the polyadenylation site, interact with CPEB, and mediate polyadenylation-induced translation in injected *Xenopus laevis* oocytes. The effect of synaptic activity on CPEB-mediated αCaMKII mRNA translation in neurons was investigated in dark-reared rats *(27).* In these animals, extensive synaptic activation stimulated by light exposure coincides with poly(A) tail elongation of αCaMKII mRNAs in the visual cortex. Furthermore, examination of biochemical synaptoneurosome preparations derived from the visual cortex revealed a light-induced translation of αCaMKII transcripts. Although additional components, such as maskin and Eg2, have not yet been identified in synaptodendritic regions, the current data suggest that CPEB is involved in the activity-dependent translational control of αCaMKII transcripts at postsynaptic sites. This assumption is further supported by two reports showing increased

synaptodendritic αCaMKII protein levels upon synaptic stimulation *(131,132).* Interestingly, the CPEs in αCaMKII transcripts do not reside in one of the functionally mapped dendritic targeting elements within the 3′-UTR *(28,29).* Thus, *cis*-elements involved in particular cellular functions do not seem to overlap. Furthermore, functional CPEs have not yet been described in other dendritically localized mRNAs implying that extrasomatic translation of these transcripts is regulated via distinct pathways.

The Multifunctional Poly(A)-binding protein, a *trans*-Factor of the Dendritic Localizer Sequence in Vasopressin Transcripts

Genes encoding vasopressin (VP) and oxytocin precursors are expressed in distinct populations of hypothalamic magnocellular neurons and transcripts from both genes are sorted into dendrites and axons *(133).* Within the VP mRNA, a 395-nucleotide spanning *cis*-acting dendritic localizer sequence (DLS) comprises the 3′-part of the coding region and the entire 3′-UTR *(30).* By UV-crosslinking analysis, the multifunctional poly(A)-binding protein (PABP) from rat brain was shown to specifically interact with the DLS, but did neither bind to the *cis*-acting dendritic targeting sequence in MAP2 transcripts nor the somatically restricted α-tubulin mRNA *(133).* An excess amount of poly(A) competitor strongly interfered with the binding of PABP to the DLS probe, whereas poly(U), poly(G), and poly(C) ribohomopolymers were ineffective. Upon purification, PABP partially looses its RNA-binding specificity. Furthermore, despite its high abundance in most tissues *(134),* a number of peripheral tissues and non-neuronal cell lines were shown to possess only minor amounts of DLS-specific PABP *(133).* These observations suggest that the specific interaction between PABP and the DLS of the VP mRNA, is in part determined by a brain-specific posttranscriptional modification of PABP or its association with additional neural proteins. PABP contains four RRMs, which are functionally diverse *(133).* The full-length protein binds with high affinity to poly(A) tails of mRNAs, enhances translation via an additional interaction with initiation factors associated with the 5′-end of mRNAs *(135),* and stabilizes transcripts in a translation-dependent manner *(136).* In vitro, PABP also interacts with sequences other than poly(A), suggesting that it may serve additional functions in mRNA metabolism *(133).* Thus, it is conceivable that PABP regulates the translation of VP transcripts and possibly other dendritically localized mRNAs.

MAP2-RNA *trans*-Acting Proteins, MARTA1 and MARTA2

In mammalian neurons, different somatodendritic isoforms of the microtubule-associated protein 2 (MAP2) regulate the stability of the dendritic cytoskeleton *(137).* MAP2 localization into dendrites appears to be a complex multicausal mechanism that involves the specific recruitment of MAP2 mRNAs into dendritic compartments *(138).* A dendritic targeting element (DTE) comprising 640 nucleotides of the 3′-UTR is both required and sufficient for effective dendritic sorting of chimeric reporter transcripts in primary neurons *(26).* Two 90- and 65-kDa MAP2-RNA *trans*-acting proteins, MARTA1 and MARTA2, specifically bind to the MAP2-DTE in vitro with nanomolar affinity *(139).* In contrast, both proteins do not interact with RNA fragments from the MAP2 coding region and the somatically restricted α-tubulin mRNA. Likewise, MARTA1 and MARTA2 do not significantly bind to other dendritically localized transcripts encoding vasopressin *(138)* and arc/arg3.1 *(20,21),* nor to dendritic trafficking elements in the 3′-UTR of the αCaMKII mRNA *(28,29,140).* Whereas MARTA1 is present in cytosolic, polysome-enriched, and nuclear fractions, MARTA2 is preferentially associated

with polysomes. Although the aforementioned data are compatible with a potential role of MARTA1 and MARTA2 in nuclear export, cytoplasmic targeting, and translation of MAP2 mRNAs, functional data are lacking. Both proteins are not restricted to rat brain, but are present in a variety of other rat tissues. Thus, MARTA1 and MARTA2 may be involved in different nuclear and cytoplasmic events regulating RNA metabolism in distinct cell types.

Summary and Extant Questions

Molecular mechanisms regulating the mRNA targeting, storage, turnover, and translation are poorly understood. This is in part due to the heterogeneous nature and vast amount of distinct mRNP particles assembled in eukaryotic cells. Considering that the human genome is estimated to contain ~1500 genes encoding RNA-binding proteins *(78),* the complexity of post-transcriptional regulation can be approximated. Although the total number of RNA-binding proteins expressed in neurons is unknown, significant progress has been made in identifying neuronal RNA-binding proteins and characterizing their putative cellular functions.

The data summarized here indicate that a growing and diverse group of RNA-binding proteins, which contain several distinct RNA-binding and protein-protein interaction domains, regulates extrasomatic mRNA targeting, translation, and stability in mammalian neurons. In vitro, these *trans*-acting factors seem to either generally bind RNA in a sequence-independent manner (Staufen), associate with a sequence motif present in a large number of differentially localized mRNAs (ELAV-like proteins), or interact very specifically with a particular *cis*-element of one or very few extrasomatically localized neuronal mRNAs (e.g., VILIP, Translin, CPEB, PABP, MARTA1, and MARTA2). Although it cannot be excluded that the further characterization of both *cis*-elements and corresponding *trans*-factors may reveal a higher degree of functional similarity between different extrasomatic messages, the current data imply that correct temporal and spatial targeting and translation of particular transcripts in neurons is established by at least partially divergent pathways.

Despite this divergence, a number of general cellular mechanisms involved in the post-transcriptional regulation of extrasomatic transcripts in neurons can be concluded from the currently available data. First, some RNA-binding proteins, such as Staufen and CPEB, appear to regulate cytoplasmic mRNA targeting and translation in different organisms and cell types as distinct as *Drosophila* and *Xenopus* oocytes and mammalian neurons. Thus, the molecular machinery underlying cytoplasmic mRNA sorting as well as a spatially restricted and locally regulated extrasomatic protein synthesis appears to be partially conserved throughout evolution. Whether these general post-transcriptional mRNA processing mechanisms, which are present in many cell types, were adapted to the specific requirements of highly polarized and excitable mammalian neurons by the addition of novel neuronal-specific factors and/or the molecular modification of more or less ubiquitous RNA-binding proteins remains to be shown. Second, similar to Staufen's dual function in cytoplasmic transcript sorting and translational derepression of *oskar* transcripts at the posterior pole of *Drosophila* oocytes, a number of the RNA-binding proteins discussed earlier appear to be multifunctional. Thus, individual *trans*-acting factors seem to fulfil distinct functions during different phases of neuronal mRNA processing. Third, a significant fraction of the mentioned RNA-binding proteins, including FMRP, ELAV- and, ZBP-1-like proteins, contain an NLS and NES and shuttle between the nucleus and the cytoplasm. Thus, these *trans*-factors are likely to bind their target mRNAs in the nucleus, mediate nuclear export, and direct distinct steps of cytoplasmic transcript processing. These findings are consistent with data obtained in various non-neuronal cell systems, in which nuclear proteins have been identified as putative *trans*-acting factors involved in cytoplasmic mRNA targeting and processing (*25,* and references therein). Fourth,

Staufen, ZBP-1-like proteins, and ELAV-containing mRNP granules associate with microtubules and/or microfilaments in vivo, indicating that cytoplasmic mRNA targeting, storage, and translation in neurons is linked to cytoskeletal filaments.

Aside from the recent progress in characterizing a number of distinct RNA-binding proteins in mammalian neurons, clear functional evidence that some of these *trans*-factors are involved in the post-transcriptional processing of extrasomatically localized transcripts is still lacking. The prospective description of neuronal mRNA targets, which are specifically associated with a particular RNA-binding protein in vivo, is like to expand significantly our understanding of the post-transcriptional regulation of mRNAs in dendrites. Furthermore, another task for the future will be to illuminate the molecular connection between synaptic signaling, the translational activation of particular postsynaptically localized transcripts, and the specific recruitment of newly synthesized transcripts to activated synaptic sites.

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