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mRNA Localization in Neurons: A Multipurpose Mechanism?

Minireview

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There is increasing evidence that an important aspect of gene expression in neurons involves the targeting of certain mRNAs to particular subcellular domains. Differential subcellular localization of mRNA is thought to provide a mechanism for local synthesis of certain proteins. Previously, the focus has been on mRNAs that were localized at postsynaptic sites and the opportunity that this provides for local synthesis of certain molecules that play a role in synaptic signaling. However, recent studies have revealed different mRNA sorting processes in immature neurons before they establish synaptic connections. These findings suggest that neurons may use the general mechanism of RNA targeting for different purposes at different times in their life history.

mRNA Localization in Dendrites

The story regarding RNA localization in neurons began with the discovery of synapse-associated polyribosome complexes (SPRCs)–clusters of polyribosomes and associated membranous cisterns that are selectively localized beneath postsynaptic sites on the dendrites of CNS neurons (for a recent review, see Steward et al., 1996). It was suggested that a particular subset of mRNAs would be localized in dendrites, allowing a local synthesis of certain proteins at postsynaptic sites.

The cloning of a host of neuronal genes coupled with the development of in situ hybridization techniques provided the means to assess mRNA localization directly. As the distributions of various mRNAs were mapped, it was established that most of the mRNAs that neurons express could be detected only in the neuronal cell body, sometimes extending for a short distance into proximal dendrites (an area that is considered to have a cytoplasmic composition similar to the soma). However, a few mRNAs were found to be present in dendrites at relatively high levels (see Steward et al., 1996). These discoveries confirmed the hypothesis that a particular subset of mRNAs was targeted to dendrites and allowed the formulation of preliminary "rules" defining RNA localization in neurons.

As the results of in situ hybridization analyses continued to accumulate, additional features of mRNA localization in dendrites became apparent. In particular, it was found that:

1) The mRNAs in dendrites encode proteins of different functional types. Among the mRNAs detected in dendrites by in situ hybridization are mRNAs that encode certain cytoskeletal proteins (the high molecular weight microtubule-associated protein MAP2 and an activity-regulated cytoskeleton-associated protein termed ARC, also known as ARG), a kinase (the α subunit of calcium/calmodulin-dependent protein kinase II), an integral membrane protein of the endoplasmic reticulum (the inositol trisphosphate receptor), calcium-binding proteins, certain subunits of neurotransmitter receptors (the α subunit of the glycine receptor, see Racca et al., 1997) as well as other proteins of unknown function (for a review, see Steward, 1996).

It is especially interesting that the mRNAs for some subunits of holomeric molecules are present in dendrites while the mRNAs for other subunits are restricted to the cell body. For example, the mRNA for the α subunit of CAMII kinase is present in dendrites at quite high levels while the β subunit is restricted to the cell body region; similarly, the mRNA for the α subunit of the glycine receptor is localized in dendrites and is even selectively localized beneath glycinergic synapses; in contrast, the β subunit is restricted to the cell body region (see Racca et al., 1997). The reasons that certain mRNAs are localized in dendrites while others are not remain obscure; however, the underlying assumption is that mRNA targeting is reserved for proteins for which local synthesis is crucial.

2) Different mRNAs are localized in the dendrites of different neuron types. One set of mRNAs is prominent in the dendrites of neurons in the cortex and hippocampus (MAP2, CAM II kinase, and ARC). A different set of mRNAs is prominent in the dendrites of cerebellar Purkinje cells (the IP_3 receptor, L7, and the calcium-binding protein PEP19, see Steward et al., 1996, for references). The differences in localization correspond to differences in the level of expression of the RNA transcripts in forebrain neurons versus Purkinje cells. An important issue is whether these mRNAs will be appropriately targeted if expressed in different neuron types.

3) Within dendrites, different mRNAs are localized in different domains. Some mRNAs are distributed throughout dendrites (CAMII kinase and ARC); others are concentrated in proximal dendrites (MAP2, see Steward et al., 1996, for references). The mechanisms that underlie this differential localization remain to be established.

Adding to the information on RNA localization have been recent studies that have revealed the presence of elements of the translational machinery within dendrites (Tiedge and Brosius, 1996) as well as elements of the RER and the Golgi apparatus (Torre and Steward, 1996). RER markers and elements of the translational machinery are present throughout dendrites; in contrast, Golgi markers are localized primarily in proximal dendrites of hippocampal neurons in culture. These results suggest that there are different capabilities for posttranslational processing within different dendritic domains. An issue that remains to be resolved is whether there is a relationship between the distribution of mRNAs for integral membrane proteins and elements of the Golgi apparatus within individual dendrites.

There is also emerging evidence that translation of dendritic mRNAs may be regulated by signaling events at synapses (Weiler and Greenough, 1993). Moreover, provocative recent studies of the mechanisms of long-term potentiation in hippocampal slices have indicated that local protein synthesis within dendrites is necessary for a form of long-term synaptic plasticity induced by

neurotrophins (Kang and Schumann, 1996). These results continue to reinforce the idea that an important aspect of synaptic function is the local synthesis of particular proteins in the subsynaptic cytoplasm. All in all, the story has interesting parallels to what has been described at the neuromuscular junction (Burden, 1993) where the mRNAs for a number of proteins, including subunits of the acetylcholine receptor, are selectively localized in the subsynaptic cytoplasm. The localization of these mRNAs appears to play a key role in the differentiation of the postsynaptic site during the development of the junction.

The discoveries on RNA localization in dendrites, together with the discovery that recently synthesized RNA is transported into dendrites but not axons of neurons in culture, suggested simple working hypotheses regarding mRNA in dendrites (see Steward and Banker, 1992): 1) under "default" conditions, mRNAs do not diffuse extensively within the cytoplasm and so remain in the cell body near their site of synthesis in the nucleus; 2) certain mRNAs contain some sort of signal that earmarks them for delivery into dendrites via a dendrite-specific transport system.

Although evidence continues to support this hypothesis for the mRNAs localized in dendrites in mature neurons, recent findings suggest that the story regarding mRNA sorting by neurons may be considerably more complicated. One line of evidence that raised new questions came from studies that provided evidence for a large number of different mRNAs in dendrites. A second arose from the discovery of mRNA in certain axon terminals. The third came from studies indicating that the rules for RNA sorting may be quite different in very young neurons.

Heterogeneity of the mRNA Pool in Dendrites

Evidence for a heterogeneous pool of mRNAs in dendrites has come from studies in which patch pipettes were used to aspirate the cytoplasmic contents of individual dendrites of neurons in culture, and then RNA amplification techniques coupled with differential display were used to clone the mRNAs (Miyashiro et al., 1994). Reverse Northern blotting techniques revealed the identity of some of the differential display products, whereas others were novel sequences.

It was especially noteworthy that the mRNAs for glutamate receptors were among the mRNAs detected. An ability to synthesize glutamate receptors at postsynaptic sites is exactly what was predicted by the original hypothesis that SPRCs synthesized key molecular constituents of the synaptic junction; but this finding presented a paradox, in that in situ hybridization analyses for various glutamate receptors have failed to detect labeling in dendrites, despite high levels of labeling in cell bodies (for a discussion, see Steward, 1994). Perhaps some mRNAs in dendrites are masked so as to interfere with hybridization. However, a more likely interpretation is that the mRNAs for glutamate receptors, as well as many of the other mRNAs that were identified by the amplification strategies are present at low levels relative to their concentration in cell bodies.

The presence of a large, heterogeneous pool of mRNAs in dendrites at relatively low levels raises an important issue. Perhaps all of the different mRNAs that were detected are selectively targeted to dendrites. If this is so, there must be a number of different dendritic targeting signals. The alternative possibility is that specific targeting is reserved for certain mRNAs–the ones that are present at high abundance relative to their levels in cell bodies, whereas the mRNAs that are present at low levels have drifted into dendrites nonspecifically.

Some "leakage" in the RNA sorting mechanisms would certainly not be surprising, given that few biological sorting mechanisms are 100% efficient, but this raises another issue. Until now, the presence of an mRNA in dendrites, however slight the level, has been considered to imply functional relevance for that mRNA localization. If certain mRNAs are present in dendrites because of a targeted delivery system, whereas others are present because of random drift, our approaches to studying mRNA localization will have to be modified. The key issue will become the relative level of the mRNA in dendrites versus cell bodies, not whether the mRNA can be detected in dendrites. Quantitative measures will have to be applied to determine relative levels, and it will be necessary to define quantitative criteria for "significant" dendritic localization.

What is clear is that a select population of mRNAs is localized in dendrites at high levels relative to their levels in cell bodies; these mRNAs are clearly sorted in a different way than other mRNAs that neurons express.

mRNAs in Axon Terminals

It is well accepted that mRNAs and translational machinery are present in the neurites of invertebrates that have the characteristics of both axons and dendrites (for a review, see Van Minnen, 1994). However, the evidence reviewed above suggested that mRNAs were in general excluded from the axons of vertebrate neurons, except for the axon initial segment, which also serves as a postsynaptic site (see Steward et al., 1996). However, in situ hybridization analyses of tissue sections have provided evidence for mRNAs in certain axon terminals in the vertebrate CNS. In particular, the mRNAs encoding the neuropeptide neurotransmitters oxytocin, vasopressin, and prodynorphin were found in high abundance in the axon terminals of the hypothalamo–hypophyseal tract (for references, see Steward et al., 1996). Also, mRNAs for the olfactory marker protein and various odorant receptors are present in the axon terminals of olfactory neurons that terminate in the olfactory bulb (Ressler et al., 1994; Vassar et al., 1994).

The significance of the localization of mRNAs in these axon terminals is not at all clear. Ribosomes and other components of the translational machinery have not yet been reported in the these axon terminals; if ribosomes are not present, then the mRNAs could not be translated. Thus, the localization of these mRNAs in axons could be incidental. The other question is whether these mRNAs are selectively targeted for delivery to axon terminals. If they are, this would mean that RNA transport is not restricted to dendrites. Alternatively, movement of these mRNAs into axons could be due to a nonspecific diffusion or transport process. Obviously, there are a number of important questions that remain to be resolved.

mRNA Localization in Immature Neurons

The evidence summarized above from studies of mRNA localization in tissue sections pertains to mature or relatively mature neurons. Studies of mRNA localization in

relatively mature neurons in culture, which had developed extensive axonal and dendritic arbors, confirmed the basic observations in tissue sections. In particular: 1) most mRNAs were restricted to neuronal cell bodies; 2) a few mRNAs, as well as ribosomes and a nontranslated polIII transcript termed BC1, were localized in dendrites, 3) mRNAs and ribosomes were not detectable in the relatively mature axons in these cultures; and 4) recently synthesized RNA was transported into dendrites but not into axons (see Steward and Banker, 1992, and references therein).

There is increasing evidence that the story may be different in very young neurons before the time that axons and dendrites exhibit their mature morphological and molecular phenotypes. In particular, in situ hybridization analyses of RNA distribution in neurons that had been maintained in culture for only a few days revealed the presence of poly(A) mRNA as well as rRNA in developing axons; especially noteworthy was the concentration of RNA in growth cones (Kleiman et al., 1994).

There are two possible interpretations of the finding that RNA is present in young axons and then disappears as the neurons mature: 1) the migration of RNA into immature axons may be incidental, reflecting the fact that the targeted transport mechanisms that mediate RNA sorting in mature neurons have not yet developed. 2) Alternatively, the RNAs in young axons may be specifically delivered into axons, where they provide a mechanism for local synthesis of certain key proteins that are important for neurite growth or guidance.

Evidence that is consistent with the latter possibility has been slowly accumulating. First, pulse-labeling techniques revealed a capability for local protein synthesis within isolated growth cones of Helisoma neurons in culture (Davis et al., 1992). Subsequently, in situ hybridization analyses revealed that poly(A) mRNA was concentrated within axonal growth cones of sympathetic neurons that were developing in culture (Olink-Coux and Hollenbeck, 1996). The latter study also provided evidence for selective mRNA transport, in that RT–PCR revealed β-actin mRNA in axons, whereas β-tubulin mRNA was detectable only in the cell body.

The latest evidence of mRNAs in growth cones has again come from studies that have used single cell mRNA amplification techniques to assay the mRNAs in dendritic growth cones of hippocamal neurons in culture (Crino and Eberwine, 1996). In this study, dendrites of young neurons (6–72 hr in vitro) were identified based on morphological and immunocytochemical criteria. The dendritic growth cone was transected, and the cytoplasmic contents were aspirated into micropipettes containing reagents necessary for single cell mRNA amplification (see Miyashiro et al., 1994). The mRNAs in the mixture were then assayed by reverse Northern blotting. A heterogeneous population of mRNAs was detected in the individual growth cones, including the mRNAs for molecules that are thought to play a role in neurite growth and guidance. Indeed, of 31 candidate mRNAs that were screened by reverse Northern blot analysis, 22 were detected in growth cones from neurons that had been cultured for 72 hr. Fewer mRNAs were detected at earlier stages.

The presence of a large, heterogeneous population of mRNAs in individual dendritic growth cones raises a number of issues. Again, there is the issue of the relative levels of the mRNAs compared to the levels in the cell body (see above). A related issue is the fact that so many mRNAs were detected. It may be that the authors were extremely astute or lucky in choosing candidates for screening. Alternatively, it could be that a large proportion of the mRNAs that the neurons express would be detectable in growth cones under these conditions. If so, this would suggest a striking lack of specificity in the RNA localization mechanisms in these young neurons. This again raises the question of whether the presence of this large, heterogeneous population of mRNAs simply indicates that young neurons have either not yet developed mechanisms to sort mRNAs properly or that mRNA sorting mechanisms were transiently disrupted when neurons were harvested and placed in culture. A key question here is whether the same population of mRNAs is present in dendritic growth cones in vivo.

The presence of a large, heterogeneous pool of mRNAs in individual growth cones also raises the issue of translational control. A key issue is the availability of ribosomes to translate the mRNAs. Electron microscopic studies of hippocampal neurons in culture indicate that although ribosomes are present in growth cones of hippocampal neurons, they are not numerous (Deitch and Banker, 1993). There must be relatively few ribosomes to go around for the large, heterogenous population of mRNAs that seems to exist. This would imply that there may be tight control over which population of mRNAs is translated at any point in time, again raising the question of whether the presence of an mRNA is sufficient to document that the localization is biologically significant.

An important contribution of the Crino and Eberwine study was the demonstration of local translation of mRNAs within growth cones, specifically local synthesis of a GSK–myc fusion protein within growth cones that had been transfected with a GSK–myc fusion reporter mRNA. In addition to providing convincing evidence that particular mRNAs can be translated within growth cones, this approach opens up new opportunities. First, it provides a potential way to assess whether local translation within growth cones is regulated by extracellular signals. Second, these results, together with recent technical advances in visualizing newly synthesized proteins within living neurons (green fluorescent protein), suggest that it may be feasible to visualize protein synthesis directly within neuronal processes. This would provide a powerful strategy to begin to define the signaling processes that regulate local protein synthesis within particular subcellular domains in neurons.

Some Caveats

There are some caveats that will be important to keep in mind as data from various model systems are brought together. It is especially important to be cautious in drawing generalizations. As noted above, mRNA localization patterns differ considerably between neuron types in vivo and under different physiological states. It is especially important to use caution in drawing general conclusions from studies of neurons in culture that have been removed from their normal tissue environment in a violent fashion and grown in a highly abnormal environment. Soon after plating, the neurons are undoubtedly still recovering from the unavoidable trauma associated

with their isolation; and as they differentiate in culture, the tissue cues that would normally influence their growth are missing. Even comparisons between similar culture systems should be drawn cautiously. Although many groups study hippocampal neurons, the neurons are cultured under very different conditions that may affect the properties of the neurons in important ways. Thus, the above argument pertaining to the stoichiometry between the number of different mRNAs and the number of ribosomes in growth cones must be considered provisional until the measures are taken in the same tissue culture setting. Obviously, drawing comparisons between neurons in vivo, in tissue slices, and in culture must be done with even more caution.

Conclusions

In mature neurons in vivo, certain mRNAs are targeted for delivery into dendrites. At least some of these mRNAs are presumably destined for the translational machinery that is selectively localized beneath synapses. Many of the mRNAs that are localized in dendrites of mature neurons encode proteins that play a role in synaptic signaling. In immature neurons in culture, mRNAs are present in axons and dendrites and are concentrated in growth cones. Some of the mRNAs in growth cones encode proteins that play a role in neurite growth and guidance. These findings suggest that neurons may use the general mechanism of RNA targeting for different purposes. In this regard, the story regarding mRNA localization in neurons continues to parallel the story regarding mRNA localization in oocytes and developing embryos, where it also appears that localized mRNAs serve multiple purposes (see St. Johnson, 1995). But studies in oocytes and developing embryos also indicate the need for caution; some examples of mRNA localization may not be biologically significant, and there appear to be examples of "gratuitous" mRNA localization. To explore this question further, it will be important to establish the purpose of RNA localization in the different situations. It is likely that the same approaches that have been used in developing systems will be useful, including disrupting the localization of particular transcripts by taking advantage of the signals that target mRNAs to particular domains. These approaches may at last help to answer the question of why neurons sort mRNAs to different subcellular domains in the first place–a question for which there is currently only speculation.

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