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Commentary

Dendrites as compartments for macromolecular synthesis

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In the past decade it has become clear that an important aspect of gene expression involves the targeting of mRNAs to different intracellular locations. This process occurs in cell types ranging from oocytes to the most spatially complex and highly differentiated cells of the body, neurons of the central nervous system. The differential subcellular localization of mRNAs determines where in the cell particular proteins can be made and with what degree of local regulation.

The story regarding neurons began with the discovery that polyribosomes are selectively localized beneath postsynaptic sites on the dendrites of a variety of central nervous system neurons (1, 2). The selectivity of the localization and the fact that synapse-associated polyribosomes are particularly prominent during periods of synapse growth (3–5) gave rise to the hypothesis that key molecular constituents of the postsynaptic junction might be synthesized on site. Obvious candidates for locally synthesized macromolecules include the molecules that make synapses operate the way that they do (neurotransmitter receptors, ion channels, and components of second messenger systems). Local synthesis of the molecular components of synapses would provide a mechanism for adjusting the macromolecular composition of individual synaptic sites based on local conditions—an appealing mechanism for synaptic plasticity.

From the beginning it was obvious that, to test these hypotheses, it would be important to identify what macromolecules were actually synthesized at synaptic sites. One obvious initial question was what mRNAs are present in dendrites. But when these hypotheses were first advanced, the question could not be addressed for technical reasons. In the first place, none of the candidate molecules had been cloned. The explosion of information over the past decade solved this problem, so that many of the genes that encode neurotransmitter receptors and other important signaling molecules of the synapse have now been cloned. But the *in situ* hybridization analyses that were done in conjunction with the cloning studies revealed that the mRNAs for neurotransmitter receptors appeared to be localized exclusively in neuronal cell bodies (see, for example, refs. 6–9). There was no significant labeling of neuropil layers in laminated structures (the cerebral cortex, cerebellum, and hippocampus), which contain dendrites but few cell bodies, so it appeared that the mRNAs for glutamate receptors were not among the ones that were present in dendrites.

Over the same period, a host of other neuronal mRNAs were cloned and their subcellular distribution was characterized. The vast majority were found to have a distribution that suggested a cell body localization, but a few mRNAs were found that had a tissue distribution that suggested a dendritic localization (i.e., labeling was present at high levels in neuropil layers in laminated structures). So far, the list includes the mRNAs encoding the high molecular weight microtubule-associated protein 2 (10), the mRNA encoding the α subunit of calcium/calmodulin-dependent protein kinase II (11), and the mRNA encoding the inositol trisphosphate type 1 receptor (12). In addition, a nonmessenger RNA of unknown function that is a polymerase III transcript (termed BC1) was also found to be present in dendritic laminae (13). The dendritic localization of some of these RNAs was subsequently confirmed by studies of neurons in culture where the degree of labeling of individual dendrites could be directly visualized (14–16). In addition, there has been one report (so far unconfirmed) that the mRNA encoding amyloid precursor protein is present in the dendrites of neurons in culture (17). Thus the story stood—that a few mRNAs were present in dendrites but that the vast majority of neuronal mRNAs were present only in the cell body.

But obviously what was needed was a systematic way to identify the full complement of mRNAs that are present in dendrites and determine their relative abundance. Addressing the question requires that several difficult technical problems be solved. First, there is the problem of selectivity—how to obtain mRNA from dendrites that is not contaminated by mRNA from neuronal cell bodies or supporting cells. Second, there is the problem of obtaining sufficient quantities of mRNA to work with. Finally, there is the problem of quantitative analysis, made more difficult by the first two problems.

The report by Miyashiro *et al.* (18) uses a collection of ingenious approaches that together provide a breakthrough to solve these problems. The problem of selectivity was solved in a simple but technically daunting way, by using patch pipettes to aspirate the cytoplasmic contents of individual dendrites of neurons grown in culture. The problems of obtaining sufficient quantities of RNA and analyzing what mRNAs were present were solved by using newly developed techniques of RNA amplification in conjunction with reverse Northern blotting. By using this array of techniques, the report provides evidence that mRNAs for glutamate receptor subunits can be detected in dendrites. If the mRNAs for glutamate receptors are associated with the polyribosomes that are selectively positioned beneath synaptic sites, then this localization would allow the synthesis of the key functional molecules of a synapse to be regulated by events at that individual synapse. It is easy to imagine how this type of mechanism might underlie activity-dependent synaptic plasticity.

The study goes on to use the technique of differential display to compare the total population of mRNAs in dendrites and cell bodies and in different dendrites from the same neuron and to clone and obtain sequence information about some of the mRNAs from dendrites. This part of the study yielded two important conclusions about the nature of the mRNAs in dendrites: (*i*) that there appears to be a substantial number of different mRNAs in dendrites, and (*ii*) that there appears to be a different blend of mRNAs in different dendrites and even different parts of the same dendrite. The nature of most of these RNAs remains unknown. Some may be messages that have not yet been cloned; others may derive from the 3′-untranslated region of mRNAs that are known but whose 3′ sequences have not been characterized. In any case, the evidence suggests that a larger number of different RNAs are present in neurites than had previously been suspected.

Taken at face value, this study provides a host of important new clues about the nature of macromolecular synthesis in dendrites and raises a number of new questions. But there are some important

concerns about the results and some puzzling contradictions that will need to be resolved. One important concern is the fact that previous *in situ* hybridization analyses have failed to detect the mRNAs for glutamate receptors in dendrites. Most previous studies evaluated RNA distribution *in vivo*, where it is possible that low but significant levels of labeling of neuropil layers might be mistaken for background. This may be more of a problem than might initially be imagined. For example, ribosomes are clearly present in dendrites (based on electron microscopic evidence), but *in situ* hybridization analyses of the tissue distribution of rRNA reveal labeling primarily over cell bodies with minimal labeling of dendritic laminae (19). The level of labeling of neuropil layers with probes to rRNA is so light that it would be impossible to ascribe that label to a specific hybridization signal without prior knowledge that ribosomes were present. At the same time, studies of RNA distribution in neurons in culture reveal that rRNA is easily detectable in dendrites (20). Thus, some mRNAs that seem to be localized only in cell bodies based on studies in tissue sections could be present in dendrites at levels that are too low to detect. However, this is not likely to account for the failure to detect the mRNAs for glutamate receptors in dendrites because *in situ* hybridization analyses of neurons *in vitro* also failed to detect dendritic labeling (21).

So what would be the significance of levels of mRNA that are below the threshold for detection using present techniques of *in situ* hybridization but that can be detected using amplification techniques (as in ref. 18)? One interpretation is that *in situ* hybridization is, in fact, not sensitive enough to detect functionally important levels of mRNA. In this regard, it will be important to reevaluate the subcellular distribution of the mRNAs encoding glutamate receptors using more sensitive *in situ* hybridization techniques as they are developed. The alternative possibility is that the amplification techniques are so powerful that they may detect mRNAs that have simply escaped the cell's normal localization mechanisms and are out of place and functionally irrelevant. The issue is, what is a functionally significant level of mRNA in any particular subcellular domain? At the moment, there is no easy resolution of this issue.

Another puzzling aspect of the results, especially given the apparently higher degree of sensitivity of the amplification technique, is that some mRNAs were *not* detected in locations where they clearly should have been. In one of the neurons, the mRNA for the glutamate receptor GluR6 was not detected in the cell body but was detected in the cell's neurites. Obviously, all eukaryotic mRNAs are synthesized in the nucleus and must pass through the perikaryal cytoplasm en route to neurites. For this reason, it is difficult to rationalize why the mRNA for GluR6 was not detected in this cell body. It may be that the amplification techniques, although extremely powerful, may still fail to consistently detect mRNAs that are present in particular domains. If so, caution would be required in interpreting the data that suggest a different blend of mRNAs in different neurites.

The results of the differential display analyses that hint at the large variety of RNAs in dendrites will likely serve as a springboard for a host of future studies. The first issue will be the nature of the proteins that the mRNAs encode. A second question will be whether any of these mRNAs can be detected in dendrites by *in situ* hybridization and if so what their subcellular distribution actually is.

The finding that a large number of different mRNAs are present in dendrites reinforces the importance of questions that have been raised previously—whether all of the mRNAs that are present in dendrites are destined for synaptic sites, whether different mRNAs are present at different types of synapses, and whether the purpose of mRNA localization is the same for different messages (22, 23). Electron microscopic studies suggest that polyribosomes in different locations have a different morphology (24); for example, they tend to have different numbers of singlet ribosomes in the clusters depending on where the clusters lie in the dendrite. It would not be surprising if some of the mRNAs in dendrites had a function that was unrelated to synapses—for example, the synthesis of dendrite-specific cytoskeletal elements (microtubule-associated protein 2). If the mRNAs for neurotransmitter receptors are among the RNAs that are translated in dendrites, it would certainly not be surprising if the mRNAs for different types of neurotransmitters were selectively localized beneath synapses of the appropriate type. The resolution of these questions will require high-resolution *in situ* hybridization analyses that are now possible because of the development of techniques that use nonradioactive probes. If there is a differential localization, the key question will be how different mRNAs are actually docked at different sites.

The presence of multiple mRNAs within individual dendrites also raises important new questions. The first question pertains to stoichiometry. If a number of different mRNAs are present in dendrites, then it would be possible to regulate the stoichiometric relationships between functionally interrelated macromolecules by regulating the synthesis of the individual proteins. This would imply a microregulation of synaptic biochemistry that is far beyond what has previously been imagined. Another important question is how the translation of different mRNAs is regulated. Localization at the synapse implies the opportunity for local regulation. Indeed, there is some evidence that neurotransmitters may regulate macromolecular synthesis within dendrites (25, 26). It will be important to determine whether the translation of different mRNAs is regulated in different ways.

And so in sum, the work by Miyashiro *et al.* (18) has opened new vistas on the story of RNA localization in neurons. Still missing, however, is a unifying hypothesis that can account for why different types of mRNA are localized in dendrites. Perhaps there are different reasons for the different transcripts. Alternatively, it may be that there are important interrelationships between the different proteins that are encoded by dendritic mRNAs that depend on local translation. Addressing these questions will require knowledge about the full complement of RNAs that are present in dendrites. It is in this regard that the work by Miyashiro *et al.* (18) may be most important in that it sets the stage for future studies that will allow a systematic cloning and identification of the transcripts generated by differential display.

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