PERSPECTIVES: NEUROSCIENCE

RNA, Whither Goest Thou?

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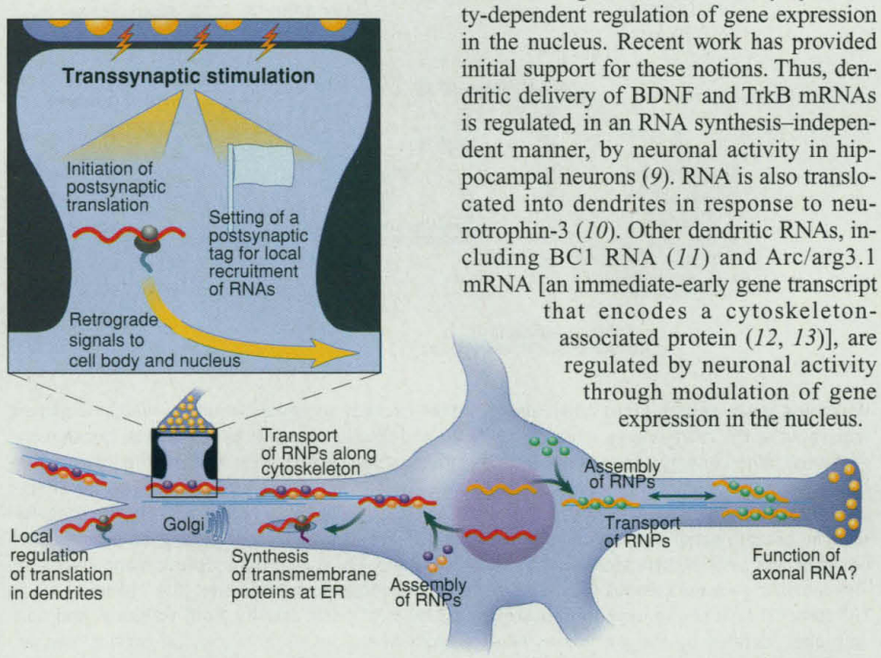
The most striking feature of nerve cells, aside from their spectacular number and the complexity of their interconnections, is the extraordinary functional plasticity of the circuitry. Each of the thousands of synaptic connections of a given neuron can be independently modulated, a modulation that requires long-term storage of specific information and thus some form of synaptic memory. We do not know whether such synaptic memory is located in the nucleus, the cell body, or at the synapse itself. If in the nucleus or cell body, a retrograde synapse-to-soma signal would be required, as would a cataloging device in the soma or nucleus to monitor the status of all of the neuron's synapses. No such device has been described to date. If long-term synaptic memory is formed at the synapse, on the other hand, a mechanism must exist to implement at least some aspects of gene expression at the synapse itself. How could this be achieved?

Enhanced online at www.sciencemag.org/cgi/content/full/283/5399/186

Recent work has advanced two answers to this question. The first suggests that stimulation of a given synapse can set a transient tag that would enable that synapse to recruit ("capture") proteins needed for synaptic modulation. Initial support for this concept has come from experiments showing that stimulation in the presence of protein-synthesis inhibitors could result in late-phase long-term potentiation provided that the same population of neurons had previously been stimulated at a different site (*1*). This is consistent with the notion that the "tagged" second site could recruit proteins that were synthesized after stimulation of the first site. However, the nature of such tags remains unknown, as do the proteins that may be recruited.

The second answer suggests that synaptic memory can be administered by the delivery of selected RNAs to postsynaptic dendritic sites (see figure at right). Select dendritic mRNAs, docked at a synaptic target site, can then be translated selectively upon demand, for example, as a result of transsynaptic activity or the action of trophic factors (*2*). In this scheme, some decision-making authority is transferred to the synapse, away from central control in the soma. A prerequisite for such mechanism is the specific transport of a subset of RNAs to dendritic sites. Various RNAs have been identified in dendrites, including mRNAs encoding neurotransmitter receptors (*2*). Dendritic RNA transport is specific and rapid—several hundred micrometers per hour (*3*, *4*)—and is mediated by cis-acting signaling elements within the transported RNAs themselves. Such elements have been mapped to the 3′ untranslated region (3′ UTR) (*5*, *6*) but also to parts of the coding region (*6*). In the noncoding short RNA polymerase III transcript BC1 RNA (152 nucleotides), a dendritic targeting element is contained within a 5′ region of no more than 62 nucleotides (*3*). No consensus sequence is apparent in such elements, suggesting that targeting competence may be determined by secondary or higher order structure motifs or by more than one motif in modular fashion.



Far from home. Dendritic and axonal RNA transport in mammalian neurons. Yellow arrows in the dendritic spine (**inset**) indicate scenarios that could result from transsynaptic activation.

The delivery of specific RNAs to dendrites would allow for localized translational regulation at the synapse. Transsynaptic activity could result in a translational switch, initiating translation of locally docked but translationally inactive mRNAs. This ability to induce the synthesis of selected proteins at the synapse would allow for long-lasting changes in structure and function of that synapse. Support for this notion comes from two recent observations: Neurotrophin-induced synaptic plasticity in the hippocampus depends on local translation in CA1 pyramidal cell dendrites (*7*), and synthesis of a rat homolog of fragile X mental retardation protein is initiated within minutes of stimulation of metabotropic glutamate receptors in synaptodendritic preparations (*8*).

Activity-dependent translation in dendrites may also be regulated by the local availability of selected mRNAs, through modulated transport or selective docking to dendritic target sites, or indirectly by activity-dependent regulation of gene expression in the nucleus. Recent work has provided initial support for these notions. Thus, dendritic delivery of BDNF and TrkB mRNAs is regulated, in an RNA synthesis–independent manner, by neuronal activity in hippocampal neurons (*9*). RNA is also translocated into dendrites in response to neurotrophin-3 (*10*). Other dendritic RNAs, including BC1 RNA (*11*) and Arc/arg3.1 mRNA [an immediate-early gene transcript that encodes a cytoskeleton-associated protein (*12*, *13*)], are regulated by neuronal activity through modulation of gene expression in the nucleus.

The dendritic localization of Arc mRNA is a direct function of synaptic input (*14*). In dentate granule cells, transsynaptic stimulation results in the selective localization of Arc mRNA only to the dendritic segments in which synapses were activated. Newly synthesized Arc protein accumulates in the same area. These data indicate that as a consequence of synaptic activation, newly synthesized mRNA can be localized to and translated at synapses that have been activated, thus providing a basis for long-lasting forms of activity-dependent synaptic modulation. On the basis of these data, the local synthesis model can

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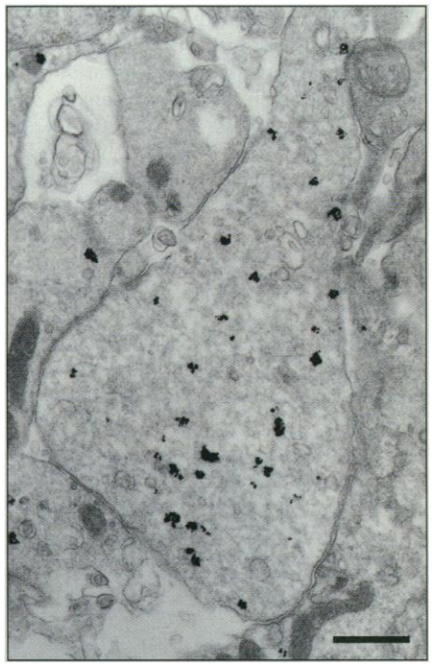
be augmented with elements of a modified synaptic tagging hypothesis in which it is RNA, rather than protein, that is being recruited by tagged synapses. Stimulation of a given synapse would thus set a tag at that site that would allow selective delivery or recruitment of dendritic RNAs to that microdomain for local translation.

A further twist has been added to this concept by a recent report that the transcription factor CREB can, upon stimulation, be translated from its cognate mRNA in dendrites and after phosphorylation be retrogradely transported to the nucleus (*15*). CREB, a regulator of gene expression in the nucleus, may thus operate as a retrograde signal linking synaptic activation to induction of gene expression. Some questions remain. What is the fate of dendritically synthesized and phosphorylated CREB (pCREB) in the nucleus? Its levels are probably quite low relative to the much larger pool of pCREB originating from other cellular localities, and it is not immediately obvious how the actions of dendritic pCREB in the nucleus could be distinct. Is pCREB of dendritic origin perhaps somehow distinct ($pCREB^d$)? Such $pCREB^d$ could then act only on a subset of CRE-containing genes (for example dendrite-relevant genes); experimental support for such a notion, however, has not yet been reported.

Do similar mechanisms exist in axons? In general, RNA transport in mature mammalian axons seems to be the exception rather than the rule and appears to be a particular feature of specialized neurons, such as magnocellular hypothalamic cells and odorant receptor cells in the olfactory epithelium. In the latter, odorant receptor mRNAs were identified in axons of olfactory neurons projecting to the olfactory bulb (*16, 17*), although it remains to be seen whether axonal expression of these receptors plays any functional role, for example, in axonal guidance mechanisms. The mRNA for tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis, can be detected in the cerebellum and the striatum. Because these regions contain no catecholaminergic cell bodies, only catecholaminergic axons, the mRNA must be present in the axons (*18*).

In magnocellular neurons, mRNA encoding the neuropeptide arginine vasopressin (AVP) has been found in axons (see figure above right), as have mRNAs for oxytocin (OT) (*19–21*), galanin (*22*), and for BC1 RNA (*23, 24*). Axonal RNA transport appears to be specific and regulated. Rats challenged by saline in the drinking water up-regulate their AVP mRNA levels 3-fold in the cell body but almost 20-fold in the axon. No relative changes in axons and cell bodies are observed for oxytocin mRNA in the osmotically stressed rats. The rates of increase and recovery for the AVP mRNA differ from the increase and recovery in the same axons for BC1 RNA (*24*). Oxytocin and AVP mRNAs are also transported to dendrites where they are colocalized with ribosomes and small secretory vesicles, suggesting that they are locally translated (*6*).

What is the function of axonal mRNAs? It is unlikely that they are translated; protein synthesis does not seem to occur in mature mammalian axons, except for the axon hillock and the initial unmyelinated segment. Axonal RNAs in mammalian neurons may, however, also serve as a retrograde signal to regulate posttranscriptional activity in the cell body. Such signaling may help to replenish a protein pool (a peptidergic neurotransmitter, for example) quickly and efficiently at times of elevated demands. Consistent with this notion are results from the mutant Brattleboro rat indicating that axonal and dendritic RNAs function quite differently. These data suggest that axonal AVP mRNA transport occurs after the message has been released from the ribosomes, whereas dendritic mRNA localization occurs independently of its association with ribosomes (*25*). The mRNA may be stably stored in the axon as part of translationally inactive ribonucleoprotein (RNP) complexes as in other cells (*26*). RNP-like particles have indeed been observed in axons of magnocellular neurons, although they could also be ribosomes (*27*). On the matter of mRNA function in mammalian axons, the jury is still out.



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Message at the end. Ultrastructural visualization of AVP mRNA within axons of the hypothalamo-neurohypophyseal system. The immunogold-silver in situ hybridization reaction is in axonal swellings of the neurohypophysis. Scale bars: 1 μm.

Important issues remain open. What is the significance of dendritic translation of a given mRNA if at the same time the encoded protein can also be targeted from the soma to synaptic sites? The recent data with Arc provide a partial answer in that synaptic activation triggers dendritic localization of the mRNA (*14*), thus allowing for activity-dependent regulation of local protein repertoires. But the advantages of dendritic translation appear less obvious with the α subunit of CaMKII, an enzyme that is distributed in neurons including dendrites. Furthermore, what are the signals that predestine a message for translation in the soma or transport to dendrites? Translation of an mRNA does not seem to be required for its dendritic transport (*14*). Do certain proteins form a complex with the mRNA, or with a pre-initiation complex, to prevent translation? If so, how would such repression then be lifted upon transsynaptic stimulation? Future research will further illuminate the significance of localized RNAs in long-term neuronal plasticity, and thus in higher brain functions.

Note added in proof. Activity-dependent polyadenylation of dendritic CaMKIIa mRNA may constitute an additional mechanism for regulating translation at the synapse (*28*).

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29. Supported by the Human Frontier Science Program Organisation (# RG-84/94B).