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- PERSPECTIVE

MOLECULAR BIOLOGY

mRNA, Live and Unmasked

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The localization of messenger RNA (mRNA) within a cell provides the opportunity for proteins to be expressed in specific subcellular compartments. This allows regions of the cell to be functionalized or modified in response to environmental cues. In neurons, long-term memory formation and synaptic plasticity require local protein synthesis at or near synapses (1). Granules comprising mRNAs and RNA binding proteins are transported within a cell, and their formation is regulated by signaling pathways (2, 3). On pages 422 and 419 in this issue, respectively, Park *et al.* (4) and Buxbaum *et al.* (5) visualize and characterize the dynamics of an endogenous mRNA. In neurons, mRNA encoding β-actin became transiently available for local translation after being released or unmasked from a latent complex. This first glimpse of endogenous mRNA behavior raises interesting questions about how RNA dynamics are coupled to translation.

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REPORT
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Adina R. Buxbaum et al.
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REPORT
Visualization of Dynamics of Single Endogenous mRNA Labeled in Live Mouse
Hye Yoon Park et al.
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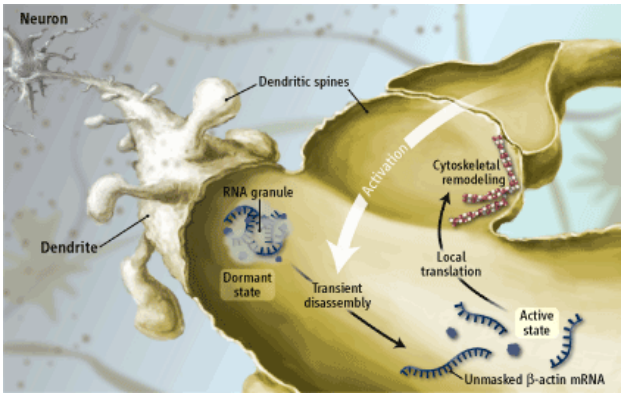
In neuronal processes and the subcompartments of other cell types, mRNAs have been detected with a variety of techniques including biochemical analysis, in situ hybridization, and RNA sequencing (6). These approaches, however, do not provide direct information on the dynamic behavior of mRNAs. To observe mRNAs live, one must associate a fluorescent molecule (either a dye or a protein) to the mRNA of interest. For example, fluorescently labeled mRNAs that are injected into cells can be analyzed for their movement, including the speed of RNA granules as well as the molecular motors and the cytoskeletal elements they use to travel (7). Delivery of molecular beacons into cells is another possibility. This technique uses a small hairpin RNA probe labeled at its ends with a fluorescent dye and a quencher, yielding a signal only when the probe is bound to the mRNA of interest. However, this approach is not commonly used for live imaging because the signal is too low. Another approach is the expression of reporter proteins that are tagged with green fluorescent protein (GFP). Such fusion proteins can bind to RNA motifs located in the untranslated region (UTR) of an mRNA of interest (8). This method has the sensitivity required to track single mRNA molecules. All of these techniques require the delivery of exogenous probes or reporters into cells, which tax cellular metabolism and often lead to cell toxicity and death (9).

Transient release.

Synaptic activity in dendritic spines triggers the release of β-actin mRNA from granules and the localized synthesis of actin, enabling cytoskeletal remodeling during plasticity.



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To circumvent some of these problems, Park *et al.* genetically engineered a mouse in which the 3′UTR of endogenous mRNA that encodes the cytoskeletal protein β-actin was designed to contain 24 stem-loop structures. This mouse was crossed with a transgenic mouse expressing multiple copies of a GFP fusion protein that binds to the stem loops. The resulting progeny expressed β-actin mRNA molecules that were fluorescently labeled. This enabled Park *et al.* to monitor the movement of endogenous β-actin mRNA in living cells.

It is a relief to see that many of the measurements previously made for β-actin mRNA dynamics appear to be reasonable. The mean transport rate in fibroblasts was 1.3 μm/s, consistent with other findings (*10*). On the other hand, although the labeled endogenous β-actin mRNAs exhibited the same types of movements (stationary, diffusive, corralled, and directed), their relative proportions were different from earlier observations (*10*). Park *et al.* noted a reduction in the number of actively transported mRNAs in fibroblasts (22% versus 1%). This may result from differences in the reporters or cell types used. Discrepancy in the ratio of motion patterns was also seen between the fibroblasts and neurons.

What is the molecular composition of an RNA granule, and how is granule composition altered by neuronal activity? Some studies have shown that multiple exogenous mRNAs may assemble into a single granule (*11*). Other studies indicate that only a single mRNA is present in a granule (*12*). In neurons, Park *et al.* observed granules containing multiple β-actin mRNA copies in the soma and proximal dendrites, but a gradual decrease to a single copy in distant dendrites. By contrast, fibroblasts contained granules with a single copy of mRNA. In neurons, RNA granules exhibited two events during their movement: merge (joining into one granule) and split (separation from a parent granule). Membrane depolarization (which causes neuron activity) increased the ratio of split to merge events, consistent with the observed increase in granules containing single β-actin mRNA as well as the overall increase in granules.

Buxbaum *et al.* examined whether the above split-merge dynamics of RNA granules occurs during synaptic plasticity to liberate mRNAs for localized expression. High-resolution imaging of mRNA particles was combined with imaging of ribosomes and ribosomal RNA (rRNA) before and after a chemical induction of neuron plasticity. The authors observed a transient increase in mRNA and rRNA in dendrites upon neuron activation. This increase was mimicked by treatment with a protease and was not prevented by a transcription blocker, which suggests that the increase resulted from unmasking of RNA from RNA binding proteins, or disassembly of granules upon neuron activation, not from mRNA that was newly transcribed in the soma and then transported to dendrites. The unmasking event correlated with an increase in local β-actin synthesis; this suggests that the mRNA is in a latent protected state in the granule, which becomes unmasked for translation when the neuron undergoes plasticity. Such a mechanism may localize the expression of β-actin in dendrites and axons to promote cytoskeletal remodeling during synaptic plasticity or axon navigation.

To what extent do the properties described by Park *et al.* and Buxbaum *et al.* apply to all localized mRNAs? There has been much discussion as to how many mRNAs are actually packaged in granules (*12*). Park *et al.* and Buxbaum *et al.* make it clear that the number of mRNAs can also change over time and in space. What function do the other constituents of the mRNA-protein complex serve if the mRNA leaves the particle? Is the granule just a storehouse equipped for translational repression, or does it have other functions? The two studies suggest that there must be elements sensitive to signaling that allow the release of an mRNA from the granule. It was recently shown that mRNAs are repressed at the elongation step of translation in the granule, waiting to be reactivated for translation, contrary to the current assumption that they are repressed at the initiation step (*13*). Future studies should elucidate the number of times a single mRNA molecule can be translated. Labeling techniques may also exert an influence on the speed, dynamics, and packing in an RNA granule. Anticipated improvements in labeling techniques, with brighter and smaller dyes, may refine our views.

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