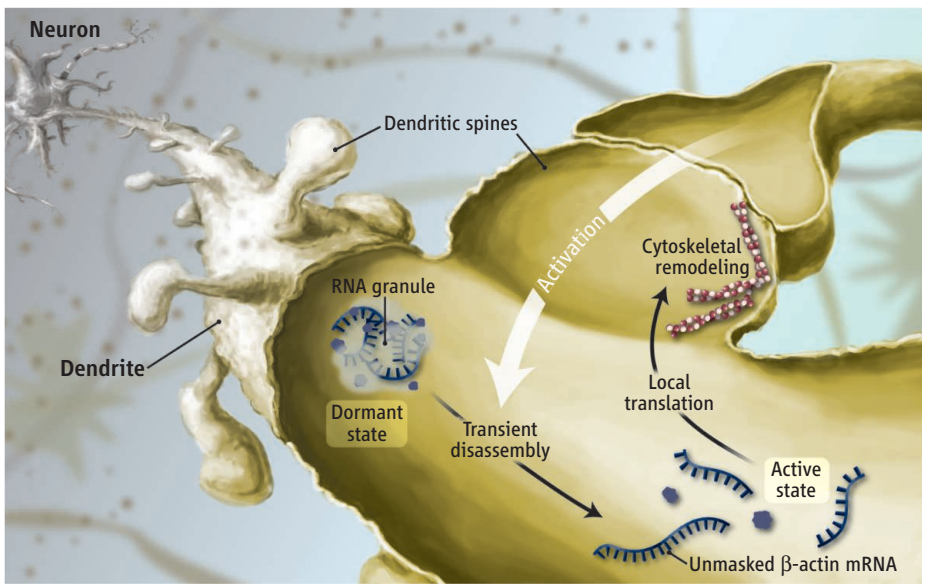
MOLECULAR BIOLOGY

mRNA, Live and Unmasked

Visualization of mRNA dynamics in live neurons reveals its release from granules at synapses during neuronal plasticity.

Güney Akbalik and Erin M. Schuman

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.



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.

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*).

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

Max Planck Institute for Brain Research, 60438 Frankfurt, Germany. E-mail: erin.schuman@brain.mpg.de

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.

References

1. C. E. Holt, E. M. Schuman, *Neuron* **80**, 648 (2013).
2. M. A. Kiebler, G. J. Bassell, *Neuron* **51**, 685 (2006).
3. M. G. Thomas, M. Loschi, M. A. Desbats, G. L. Boccaccio, *Cell. Signal.* **23**, 324 (2011).
4. H. Y. Park *et al*., *Science* **343**, 422 (2014).
5. A. R. Buxbaum *et al*., *Science* **343**, 419 (2014).
6. I. J. Cajigas *et al*., *Neuron* **74**, 453 (2012).
7. G. S. Wilkie, I. Davis, *Cell* **105**, 209 (2001).
8. E. Bertrand *et al*., *Mol. Cell* **2**, 437 (1998).
9. T. T. Weil, R. M. Parton, I. Davis, *Trends Cell Biol.* **20**, 380 (2010).
10. D. Fusco *et al*., *Curr. Biol.* **13**, 161 (2003).
11. F. Tübing *et al*., *J. Neurosci.* **30**, 4160 (2010).
12. M. Batish, P. van den Bogaard, F. R. Kramer, S. Tyagi, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 4645 (2012).
13. T. E. Graber *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **110**, 16205 (2013).

10.1126/science.1249623

CANCER

Hiding in Plain View—An Ancient Dog in the Modern World

The sequence of a transmissible cancer provides a snapshot of a dog from the distant past.

Heidi G. Parker and Elaine A. Ostrander

A long-ago ancestor of the modern domestic dog is alive today in the form of the canine transmissible venereal tumor (CTVT). This tumor was first identified in the late 1800s when it was found to be transferred to new hosts through tumor cells (*1*). We now know that the tumor is naturally transmitted between dogs by direct contact, primarily through coitus or activities that permit sloughing of cells (*2*). The tumors are rarely metastatic and most tumors regress within a few months, leaving previously infected dogs with immunity. DNA analysis provides strong evidence that all CTVTs studied thus far are from a single source, one that existed before the dispersal of dog breeds around the world (*3*, *4*). On page 437 of this issue, Murchison *et al*. (*5*) describe the first whole-genome sequence of CTVT, sampled from two tumors: one from a random-bred Australian Aboriginal camp dog and the other from a purebred American cocker spaniel from Brazil.

Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892–8000, USA. E-mail: eostrand@mail.nih.gov

Sequence analysis of the two tumors revealed ~1.7 million somatic variants shared between them. These variants are presumed to have arisen during the initial malignant transformation or in the years of tumor passage before separation of the tumors by geographic boundaries. The number of somatic mutations is >100 times as large as the average mutation load of a human tumor, highlighting the long period over which mutations have accumulated and the number of alterations required to develop a stable colony of tumor cells. Mutations were scattered throughout the genome with more than 10,000 genes carrying at least one predicted protein-modifying genetic change. This list encompasses nearly half of the annotated genes from the dog reference sequence (*6*) and illuminates a cadre of genes and proteins necessary for cellular replication, maintenance of the tumor phenotype, and pathogenicity.

An examination of 23,782 single-nucleotide polymorphisms in both CTVTs and 1106 modern dogs and other canids places the origin of the tumor at ~11,000 years before present, with a second divergence of geographical tumor strains occurring <500 years ago. Principal component analysis suggests that the first dog with CTVT was a member of the ancient dog group, the dog breeds closest to the wolf (*7*). Pairwise distance calculations place the Alaskan Malamute, a 4000-year-old breed that originally came to America from Eastern Asia, as the closest living relative of CTVT. In addition, the founder animal carried a mixture of "wolflike and doglike" alleles and was likely medium to large in size with short, straight fur, a black or agouti coat, prick ears, and a meso- to dolicocephalic head shape (see the figure). Dog fanciers will note that this description fits the modern Alaskan Malamute, but could also match that of any number of large spitz-type dogs.

Naturally occurring transmissible tumors are extremely rare. The only other known example is the Tasmanian devil facial tumor disease (DFTD), which was first identified in 1996 and has spread rapidly throughout the devil population. Unlike the canine tumor, DFTD is highly virulent, metastasizes readily, and is ultimately fatal (*8*). Sequencing of DFTD revealed a three- to eightfold increase in somatic mutations compared to human tumors as opposed to the >100-fold increase