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- REPORT

Visualization of Dynamics of Single Endogenous mRNA Labeled in Live Mouse

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ABSTRACT | EDITOR'S SUMMARY

The transcription and transport of messenger RNA (mRNA) are critical steps in regulating the spatial and temporal components of gene expression, but it has not been possible to observe the dynamics of endogenous mRNA in primary mammalian tissues. We have developed a transgenic mouse in which all β-actin mRNA is fluorescently labeled. We found that β-actin mRNA in primary fibroblasts localizes predominantly by diffusion and trapping as single mRNAs. In cultured neurons and acute brain slices, we found that multiple β-actin mRNAs can assemble together, travel by active transport, and disassemble upon depolarization by potassium chloride. Imaging of brain slices revealed immediate early induction of β-actin transcription after depolarization. Studying endogenous mRNA in live mouse tissues provides insight into its dynamic regulation within the context of the cellular and tissue microenvironment.

Recent advances have provided insights into the behavior of RNA in real time (*1*). However, most live-cell imaging techniques require transfection or injection of exogenous reporters that are typically overexpressed or are missing regulatory elements and binding partners present in the endogenous molecules. Moreover, immortalized cells may not accurately exhibit RNA regulation representative of the native tissue environment.

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To address these limitations, we generated a transgenic mouse in which all endogenous β-actin mRNA is fluorescently labeled by specific binding between the MS2 bacteriophage capsid protein (MCP) and the MS2 binding site (MBS) RNA stem-loops (*2*). Lentiviral transgenesis (*3*) was used to integrate the MCP-GFP (green fluorescent protein) transgene with high efficiency (fig. S1). The resulting MCP mice were crossed with Actb-MBS mice, in which 24 repeats of MBS are knocked into the 3′ untranslated region (UTR) of the β-actin gene (*4*), to label endogenous β-actin mRNA with GFP, resulting in MCP×MBS mice (**Fig. 1A**). β-Actin mRNA, which is essential for early embryonic development (*5*), was labeled with the 1200-nucleotide MBS cassette and up to 48 molecules of MCP-GFP (fig. S2A), yet no abnormalities were found by histologic analysis (n = 3 mice); mice were fertile (fig. S2B), and β-actin mRNA and protein expression levels were similar to those of wild-type mice (fig. S2, C and D). Hence, MS2-GFP labeling of endogenous β-actin mRNA did not disrupt its function and the expression level in vivo, confirming the physiological relevance of the studies.

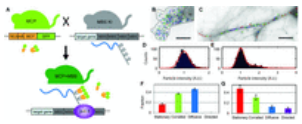

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Fig. 1. Labeled endogenous mRNA in MCP×MBS mouse.

(**A**) Schematic for in vivo RNA labeling. NLS, nuclear localization sequence; HA, hemagglutinin; pol II, RNA polymerase II. (**B** and **C**) Single-particle tracking of GFP-labeled β-actin mRNP in primary MEF (B) and hippocampal neuron (C) from mouse. Track classifications: red, stationary; green, corralled; blue, diffusive; purple, directed motion (see supplementary text). N, nucleus; C, cytoplasm; S, soma. Scale bars, 10 μm. (**D** and **E**) Intensity histograms of β-actin mRNPs tracked in primary MEFs (D) and neurons (E). Red curves show one- and three-component Gaussian fits for (D) and (E), respectively. (**F** and **G**) Fraction of stationary, corralled, diffusive, and directed β-actin mRNPs in primary MEFs (F) and neurons (G). Error bars denote SEM (n = 6 cells).

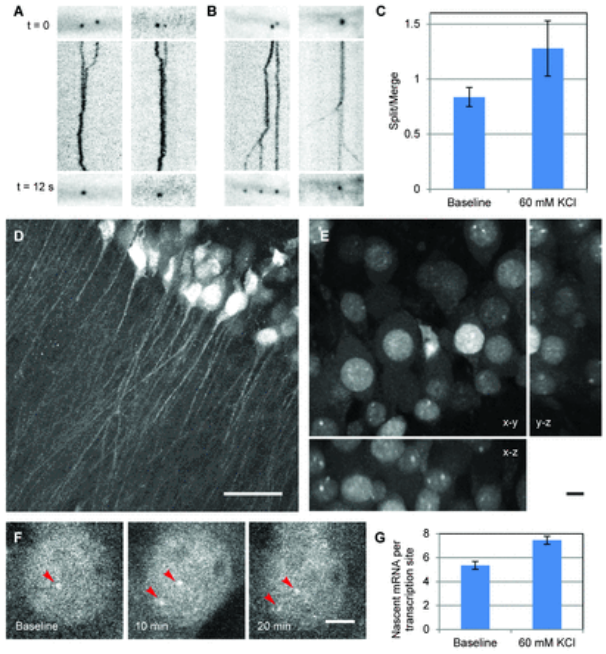
Localization of β-actin mRNA was observed in primary fibroblast leading edges (*6*), neuronal growth cones (*7*), and mature neuron dendrites and spines (*8*) by fluorescence in situ hybridization (FISH). However, it is unclear how β-actin mRNA localizes in real time. Because loss of mRNA localization occurs in culture, we imaged mouse embryonic fibroblasts (MEFs) from MCP×MBS mice within 48 hours after isolation (**Fig. 1B**, fig. S3, and movie S1). Individual mRNA-protein complex (mRNP) particles were identifiable in MCP×MBS cells, unlike the background in MCP cells (fig. S2, E and F). Both single-molecule FISH and GFP labeling in live cells showed that these particles contained only single copies of β-actin mRNA (**Fig. 1D**, fig. S4, and supplementary text). The ensemble diffusion coefficient of the labeled endogenous β-actin mRNA was 0.09 ± 0.02 $\mu m^2/s$, similar to a reporter mRNA in a cell line (*9*). However, the movement patterns of endogenous mRNA appeared different from those of exogenous mRNA. There was less directed motion (~1%) of endogenous mRNA (**Fig. 1F** and fig. S5) than previously reported (22%) (*9*), possibly due to the differences between the endogenous mRNA and the exogenous reporter, or the cell types. Serum-induced localization of β-actin mRNA in fibroblasts appears predominantly mediated by rapid release of stationary mRNA and redistribution into discrete cytoplasmic compartments (fig. S6, movies S2 to S5, and supplementary text), although we cannot rule out short movements driven by nonprocessive motors.

Neuronal RNA transport granules may contain multiple mRNAs (*10*, *11*). To investigate the stoichiometry of β-actin mRNA in hippocampal neurons from MCP×MBS mice, we performed single-molecule FISH (fig. S7). The intensity histograms of diffraction-limited fluorescent spots indicated mRNPs containing multiple copies of β-actin mRNA in the soma and proximal dendrites (fig. S7B), which decreased with distance from the soma (fig. S7D). In live neurons (**Fig. 1C** and movie S6), ~25% of mRNPs in proximal dendrites contained more than one β-actin mRNA (**Fig. 1E**). Diffusion of mRNPs in neurons was slower [diffusion coefficient = 3.8 (±0.5) × 10^{-3} $\mu m^2/s$] than in fibroblasts, but ~10% of mRNPs were actively transported anterograde and retrograde (**Fig. 1G** and fig. S8A) with a mean speed of 1.3 μm/s (fig. S8B). The ratio of anterograde to retrograde transport was 1.1 to 1.5 throughout neuronal development in culture (fig. S8C), which may mediate constitutive delivery into dendrites.

To investigate the activity-dependent dynamics of β-actin mRNA, we imaged live neurons before and after depolarization (60 mM KCl for 3 to 6 min). Pairwise comparisons in the same dendritic regions revealed that there were significant increases in the density of the mRNP particles after KCl depolarization in both cultured neurons (fig. S9) (*8*) and acute brain slices (fig. S10). The diffusion coefficient decreased by a factor of 3 (fig. S9D), and particles with directed motion decreased in both directions (fig. S9E). Therefore, the increase of diffraction-limited spots in the dendrite was not due to transport of mRNA from the soma. We hypothesized that the number of detected spots increased because of the release of mRNAs from mRNP complexes upon stimulation. We quantified the number of β-actin mRNAs contained in each neuronal mRNP by particle intensity. After KCl depolarization, the number of spots containing single β-actin mRNA increased while the number of particles bearing multiple mRNAs decreased (fig. S9B and S10B). We observed merge and split events of particles (**Fig. 2, A and B**, and movies S7 and S8). Both the split and merge frequencies were reduced after depolarization, but the ratio of split to merge was increased (**Fig. 2C**). These results suggest that mRNA molecules undergo continuous assembly and disassembly of large mRNP complexes (*12*) but favor the released state upon depolarization, possibly for local translation (*13*, *14*).

Fig. 2. Activity-dependent dynamics of β-actin mRNP in neurons from MCP×MBS mouse.

(**A** and **B**) Examples of β-actin mRNP merge (A) and split (B) events. From top to bottom: initial images of the particles, kymographs during a 12-s period, and final images. (**C**) Ratio of split to merge events increased after KCl depolarization (n = 11 neurons from three cultures, 14 to 16 days in vitro; $P < 0.05$ by t test). (**D**) CA1 region in acute hippocampal slice. Scale bar, 50 μm. (**E**) Soma layer of CA1 neurons. Panels show maximum projections of x–y, y–z, and x–z planes. Scale bar, 10 μm. (**F**) Transcription of β-actin gene with KCl depolarization. Arrowheads denote β-actin transcription sites. Scale bar, 5 μm. (**G**) Nascent mRNA per transcription site increased after



depolarization (n = 86 sites, brain slices from 3 mice; $P <$ 0.001 by pairwise t test). Error bars denote SEM.

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We investigated endogenous β-actin gene expression in native tissue by imaging acute brain slices (**Fig. 2D**). Transcriptional activity was monitored in the hippocampus CA1 region at 20 to 60 μm from the surface before and after KCl depolarization (**Fig. 2E**). Nascent β-actin mRNA per transcription site increased 10 to 15 min after depolarization (**Fig. 2, F and G**), likely because of rapid initiation (*15*). Rapid induction of β-actin transcription was observed in various cell lines (*4*, *16*, *17*) but β-actin was not recognized as an immediate early gene in the nervous system, probably because of high basal expression. Increased expression of β-actin may be implicated in transducing synaptic activity into structural plasticity.

The MCP×MBS mouse provides a distinctive tool for monitoring the dynamics of single endogenous mRNA in live mammalian cells and tissues. Our results with the β-actin gene suggest that the technique could be generally applicable to other genes to investigate the effect of the tissue microenvironment on single-cell gene expression.

Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S10

Movies S1 to S8

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