Activity-dependent spatially localized miRNA maturation in neuronal dendrites

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MicroRNAs (miRNAs) regulate gene expression by binding to target messenger RNAs (mRNAs) and preventing their translation. In general, the number of potential mRNA targets in a cell is much greater than the miRNA copy number, complicating high-fidelity miRNA-target interactions. We developed an inducible fluorescent probe to explore whether the maturation of a miRNA could be regulated in space and time in neurons. A precursor miRNA (pre-miRNA) probe exhibited an activity-dependent increase in fluorescence, suggesting the stimulation of miRNA maturation. Single-synapse stimulation resulted in a local maturation of miRNA that was associated with a spatially restricted reduction in the protein synthesis of a target mRNA. Thus, the spatially and temporally regulated maturation of pre-miRNAs can be used to increase the precision and robustness of miRNA-mediated translational repression.

MicroRNAs (miRNAs) regulate gene expression by binding to the 3′ untranslated region (3′UTR) of target mRNAs and preventing their translation (1). A mature miRNA is generated by the sequential processing of two different precursors: the primary miRNA (pri-miRNA) and the precursor miRNA (pre-miRNA). The pri-miRNA is processed in the nucleus (2) and then exported to the cytoplasm, where it is processed by Dicer (3) to generate the mature miRNA. Bioinformatics allow the prediction of individual miRNA binding sites across transcripts (4, 5), suggesting that each miRNA can target multiple transcripts (6). The copy number of most miRNAs is relatively low (7, 8) when compared with the copy number and abundance of mRNA targets (9), raising the question of how a miRNA can effectively regulate a particular target mRNA. One means to enhance the efficiency of regulation would be to promote the spatial proximity of the miRNA and its target mRNA, thus increasing the probability of miRNA-target interactions. This spatial colocalization could be accomplished by regulating miRNA maturation—that is, by first localizing the pre-miRNA and then controlling its maturation in both space and time.

We tested this idea in neurons, where local translation (10) and miRNAs (7, 11–15) have been reported to regulate synaptic function in morphologically complex axons and dendrites. To determine the number of potential mRNA targets that dendritic and axonal miRNAs might bind, we evaluated the expression and abundance of miRNAs in the hippocampal neuropil by using NanoString (16) (Fig. 1A and table S1) and then identified the number of potential dendritic mRNA targets, using 3′UTR isoform sequences from neuropl-localized miRNAs (Fig. 1B) (e.g., 17). For a given neuronal miRNA, we detected a broad range in the number of potential targets, ranging from 31 to 1077, with a median of 503. If a pre-miRNA is processed locally, then both the precursor and the machinery required for processing should be present in the dendrites. Dicer (18) and pre-miRNAs have been observed in dendrites and near synapses (19–21). We found that the precursor for miR-181a, a neuronal miRNA that is highly expressed in the hippocampus (Fig. 1A), is present in both the cell bodies and dendrites of cultured hippocampal neurons and hippocampal slices (Fig. 1, C to F, and fig. S1). When compared with mature miR-181a, there is a greater abundance of pre–miR-181a in the cell bodies, but a roughly equal number of particles in the neuropil (fig. S2).

To determine whether there is active processing of pre-miRNA in dendrites, we made an inducible fluorescent sensor (22), using the structure of pre–miR-181a as a backbone (Fig. 2A). A fluorophore was placed in the double-stranded backbone of the miRNA, and a quencher was added to the loop region. In the intact pre–miR-181a probe, the fluorophore was quenched. When Dicer processed the pre-miRNA, the loop region was cleaved, liberating the quencher and enabling the measurement of fluorescence. Endogenous Dicer activity was sufficient to stimulate probe cleavage in a cellular lysate prepared from control or conditional Dicer knockout mice (23) (Fig. 2B). In Argonauta pull-down experiments from hippocampal lysates spiked with the pre–miR-181a probe, we observed a significant increase in fluorescence associated with Argonauta, relative to a control pull-down (Fig. 2C). Thus, the probe works as designed to report the Dicer-dependent cleavage of pre–miR-181a.

We introduced the fluorescent probe to individual hippocampal neurons via a patch pipette together with a spectrally distinct fluorescent dye (Alexa 488) (Fig. 2E). Under basal conditions, the processing of the pre-miRNA probe, as indicated by an increase in probe fluorescence over time, was evident primarily in the cell body over tens of minutes (Fig. 2, E, G, and H). To examine whether the maturation of pre–miR-181a is sensitive to neural activity, we elicited action potentials (Fig. 2D) and continued to monitor the probe fluorescence (Fig. 2F). Depolarization led to a dramatic increase in pre-miRNA maturation, as indicated by an increase in fluorescence observed in both the soma and dendrites (Fig. 2, F to H). In neurons in which Dicer expression levels were knocked down by using a short hairpin RNA (figs. S3 and S4D), the activity-induced fluorescence increase was significantly reduced (Fig. 2, G and H, and fig. S5). Calcium can stimulate Dicer activity (21), and increases in some miRNAs have been observed after activation of NMDA (N-methyl-D-aspartate) receptors (23, 24). To examine whether an activity-induced increase in intracellular Ca²⁺ (mediated by NMDA receptors) is important for pre-miRNA maturation, we conducted experiments in the presence of a NMDA receptor antagonist, APV (D,L-2-amino-5-phosphonovaleric acid; 50 μM) (Fig. 2, G and H). NMDA receptor antagonism completely prevented the stimulation-induced increase in the pre–miR-181a probe and reduced the basal level of probe maturation observed in dendrites (Fig. 2, G and H).

These data indicate that neural activity can stimulate the Dicer-dependent maturation of pre-miRNAs. To examine whether local synaptic activity regulates the maturation of pre-miRNAs, we conducted glutamate uncaging experiments to control the activation of individual synapses. Cultured hippocampal neurons were bathed in caged glutamate, and an Alexa dye and the pre–miR-181a probe were introduced into neurons as before (fig. S4, A and B). A dendritic region was chosen for uncaging (Fig. 3A) on the basis of the ability of test uncaging pulses to elicit a current (uncaging-evoked excitatory postsynaptic current, uEPSC) (average uncaging spot size, 2 μm²; Fig. 3B). While monitoring the probe fluorescence in the patched neuron, a train of uEPSCs was evoked (~1 Hz, 20 pulses) by uncaging glutamate in a region close to the dendrite (fig. S4C). Coincident with the activation of a single synapse, a rapid and spatially localized fluorescence increase was observed in the dendritic shaft immediately adjacent to the stimulated area (Fig. 3, C and D) or, in some cases, directly in the dendritic spine, suggesting the generation of a mature miRNA (Fig. 3, E and F). The probe fluorescence occurred with an onset that was slightly variable with respect to the start of the uncaging train, ranging from ~1 to 5 s, and began as a ~1-μm² spot that...
Fig. 1. Pre-miR-181a is present in neuronal dendrites. (A) Graph (means ± SEM) showing all miRNAs detected in the neuropil of area CA1 of the rat hippocampus (purple dots; 138 of 443 present in the code set) by using nCounter NanoString (for 3; stars, positive spike-in; black dots, negative spike-in; gray dots, not detected (305 of 443)). (B) The predicted number of target mRNAs (y axis) for all miRNAs detected in the hippocampal neuropil (A). The median and quartiles are shown. (C) Representative in situ hybridization image (and magnification of one dendrite, right panel) acquired using a probe directed against the loop region of pre-miR-181a. The median and quartiles are shown. (D) Scrambled control for in situ hybridization. Scale bars in (C) and (D), 10 μm. (E) Representative in situ hybridization image of pre-miR-181a in the CA1 region of a hippocampal slice (and magnification of the boxed dendritic region, right panel). The in situ hybridization signal is abundant in the soma and neuropil layer. (F) Very low signal is observed in the scrambled control. Scale bars in (E) and (F), 50 μm. DAPI, 4’,6-diamidino-2-phenylindole.

Fig. 2. An inducible probe reports the activity-dependent maturation of a miRNA. (A) Schematic of the pre–miR-181a probe showing the fluorophore (F) and quencher (Q) near the base of the loop. (B) In control lysates, the fluorescence generated by probe processing was significantly higher than that observed in Dicer KO lysates (see the methods section in the supplementary materials; ***P < 0.0001, n = 3 animals). (C) Probe fluorescence after immunoprecipitation (IP) with either an Argonaute 2 (AGO2) or immunoglobulin G (IgG; control) antibody (one experiment with four and two replicates for AGO2 and IgG, respectively). (D) Representative whole-cell recording showing intracellular current injection and action potentials. (E) Alexa dye–filled recorded neuron (left) and time-lapse images of pre–miR-181a probe fluorescence over time (right). An increase in probe fluorescence in the soma (white dotted line) was detectable over the course of 25 min, presumably reflecting basal levels of Dicer activity. Scale bar, 20 µm. (F) Stimulated Alexa dye–filled recorded neuron (left) and time-lapse images of probe fluorescence over time (right). At 7 min, the neuron was depolarized, leading to a rapid increase in probe fluorescence in the soma (white dotted line) and dendrites. Scale bar, 20 µm. (G) Group analysis (somata) showing the significant increase in probe fluorescence after stimulation (*P < 0.05; one-way repeated measures analysis of variance (rANOVA)), which was blocked by Dicer knockdown (**P < 0.05; one-way rANOVA) or a block of NMDA receptors (APV; 50 µM) (**P < 0.05; one-way ANOVA). (H) Group analysis (dendrites) showing the significant increase in probe fluorescence after stimulation (**P < 0.05; one-way ANOVA); either Dicer knockdown or APV reduced the basal maturation of the probe (**P < 0.05; one-way ANOVA) and blocked the stimulation-induced increase (*P < 0.05; one-way ANOVA) (n = 10, 8, 8, and 7 for basal, stimulated, stimulated + Dicer KD, and stimulated + APV groups, respectively). Arrows indicate the time of stimulation. All values are means ± SEM.

Fig. 3. Local stimulation leads to the rapid and spatially restricted maturation of miR-181a in dendrites and spines. (A) Alexa dye–filled recorded neuron. The yellow box shows the dendritic segment in (C) with the glutamate uncaging spot. Scale bar, 10 µm. (B) Representative spontaneous excitatory postsynaptic currents (sEPSCs) and uncaging-elicited currents (uEPSCs). (C) Time-lapse images of pre–miR-181a probe fluorescence before and after local glutamate uncaging (yellow arrow). An increase in fluorescence was evident within 1 s after uncaging. Scale bar, 2 µm. (D) Group analysis of dendrites showing the significant uncaging-induced increase in probe fluorescence (**P < 0.0001; two-way ANOVA), which was blocked by Dicer knockdown or a block of NMDA receptors (APV; 50 µM). In some cases, error bars are small and occluded by the symbol. Photostimulation without caged glutamate did not produce a fluorescence increase (n = 23, 6, 6, and 5 for uncaging, Dicer KD, uncaging + APV, and laser-only groups, respectively). (E) Time-lapse images of probe fluorescence in a dendritic spine during local glutamate uncaging. Scale bar, 2 µm. (F) Group analysis of neuronal dendritic spines showing the significant increase in probe fluorescence after local glutamate uncaging (**P < 0.0004, n = 6; paired t test). All values are means ± SEM.
Fig. 4. The local activity-dependent maturation of miR-181a is associated with a decrease in the translation of a target mRNA, CamKIIu. (A) Alexa dye-filled recorded neuron showing the glutamate uncaging spot (small yellow box indicated by the white arrow). Scale bar, 10 μm. (B) Time-lapse image of probe fluorescence in a dendritic spine during local glutamate uncaging (yellow box, uncaging spot). In this experiment, the Alexa spine signal increased as a result of uncaging, owing to either changes in spine morphology or diffusion of the dye into the spine head. Scale bar, 5 μm. (C) Representative image showing newly synthesized CamKIIu particles after labeling and glutamate uncaging (arrow, region of uncaging). (D) Higher magnification of the boxed area in (C) (green, Puro-PLA signal for nascent CamKIIu; blue, dendrites immunostained with antibody against MAP2; white box, uncaging spot). A paucity of newly synthesized CamKIIu particles is observed in the region of uncaging, as well as the adjacent dendritic shaft. Scale bars in (C) and (D), 20 μm. (E) Summary graphs indicating the mean fluorescent intensity obtained in the proximal and uncaged dendritic segments (see the methods; n = 6 pairs of dendrites, **P = 0.0054). In (E) to (H), uncaged and within-neuron control dendrites are shown in green and gray, respectively. (F) Schematic (top) and analysis (bottom) of newly synthesized GFP particles in neurons transfected with the GFP-CamKIIu long 3’UTR construct after labeling with puromycin and glutamate uncaging. (n = 9 pairs of dendrites, **P = 0.0017). (G) Schematic (top) and analysis (bottom) of newly synthesized GFP particles in Dicer knockdown neurons transfected with the GFP-CamKIIu 3’UTR construct. (n = 6, P = 0.84, not significant (ns)). (H) Schematic (top) and analysis (bottom) of newly synthesized GFP particles in neurons transfected with a construct lacking the miRNA seed region in the 3’UTR (n = 6, P = 0.57, ns). All P values are from unpaired t tests. Bars represent means (longer bars) and SEM.

which the potency and robustness of miRNA regulation of gene expression can be achieved.

REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S9

Table S1

References (31–36)

Movies S1 and S2

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Editor's Summary

Intraneuronal control of protein expression

In cells and tissues, mRNA copy numbers far exceed the number of micro RNAs (miRNAs). How then can a miRNA effectively regulate translation of a particular target mRNA? Sambandan et al. used high-resolution in situ hybridization to detect precursor miRNA in rat neuronal dendrites. They introduced a fluorescent miRNA maturation reporter into hippocampal neurons and detected activity-dependent maturation of the probe in both the soma and dendrites. This local maturation of the miRNA was indeed associated with a local reduction in protein synthesis. Thus, localized miRNA maturation can modulate target gene expression with local and temporal precision.

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