Cellular/Molecular

Modulation of mGluR-Dependent MAP1B Translation and AMPA Receptor Endocytosis by MicroRNA miR-146a-5p

Ying-Lin Chen[1,2] **and Che-Kun James Shen**[1,2]

[1]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei 112, Taiwan, Republic of China and [2]Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan, Republic of China

The translation of dendritic microtubule-associated protein 1B (MAP1B) is exaggerated upon group I mGluR activation leading to AMPA receptor (AMPAR) endocytosis and consequent long-term depression. However, the mechanisms of regulation of MAP1B protein synthesis in the mature dendrites remain unclear. Here we have identified miR-146a-5p that targets the 3′ UTR of MAP1B mRNA and represses its translation. Inhibition of the endogenous miR-146a-5p in mouse cultured hippocampal neurons triggers an increase of the dendritic MAP1B protein as well as the internalization of AMPARs, resulting in a decline in synaptic transmission. Conversely, enforced expression of miR-146a-5p inhibits MAP1B translation and attenuates group I mGluR-induced AMPAR endocytosis. Moreover, siRNA-mediated knockdown of MAP1B recovers the impairment of synaptic transmission caused by inhibition of miR-146a-5p. These results reveal that miR-146a-5p modulates the synaptic plasticity via repression of MAP1B protein synthesis.

Introduction

The regulation of synaptic plasticity, which is fundamental for learning, memory, and cognition in response to neuronal activities, involves the control of translation of dendritic proteins (Sutton and Schuman, 2005). The microtubule-associated protein 1B (MAP1B) appears to play a regulatory role in synaptic plasticity. The protein is distributed in the dendrites of mature neurons with localization in the postsynaptic densities (Schoenfeld et al., 1989; Kawakami et al., 2003). The distribution of MAP1B protein in the plasma membrane fraction is increased during synaptogenesis (Kitamura et al., 2007). Fitting the scenario that metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) relies on rapid synthesis of postsynaptic proteins (Huber et al., 2000), stimulation of group I mGluRs with (*RS*)-3,5-dihydroxyphenylglycine (DHPG) induces the translocation of MAP1B mRNA from the soma into the dendrites (Dictenberg et al., 2008) and its translation (Hou et al., 2006; Davidkova and Carroll, 2007). Simultaneously, the MAP1B–GRIP interaction is enhanced and it stabilizes the internalized AMPA receptors (AMPARs) (Braithwaite et al., 2002; Davidkova and Carroll, 2007), thus leading to LTD (Snyder et al., 2001; Collingridge et al., 2010). However, the regulatory mechanisms of MAP1B translation in the mature dendrites have remained elusive.

MicroRNA (miRNAs) are short (19–25 nucleotides) endogenous RNAs regulating a diverse range of biological functions, including synaptic plasticity (Olde Loohuis et al., 2012), through interference of translation and/or mRNA degradation (Bartel, 2004; Kim, 2005; Wienholds and Plasterk, 2005). We present evidence that expression of MAP1B protein, mGluR-induced MAP1B augmentation, and AMPAR endocytosis in mature neurons are all regulated by miR-146a-5p, a miRNA dysregulated in Rett syndrome (RTT) (Urdinguio et al., 2010). Furthermore, the targeting of miR-146a-5p to a specific region of the MAP1B mRNA mediates MAP1B protein synthesis through translational repression. Inhibition of miR-146a-5p results in a decrease in the frequency of miniature EPSCs (mEPSCs), which is restored by the siRNA knockdown of MAP1B. Thus, miR-146a-5p is likely one of the key factors guarding against AMPAR internalization and consequent LTD through repression of excessive MAP1B protein synthesis.

Materials and Methods

Antibodies and chemicals. The MAP1B (GTX116014) and GAPDH antibodies (GTX100118) used in the Western blotting were purchased from GeneTex. Antibodies against MAP1B (N-19; sc-8970; Santa Cruz Biotechnology), GluA1 (PC246; Calbiochem), and MAP2 (clone HM-2; M9942; Sigma-Aldrich), respectively, were used in the immunofluorescence staining experiments. All fluorescence-conjugated secondary antibodies were purchased from Invitrogen. DHPG (0342), D-(−)-2-amino-5-phosphonopentanoic acid (AP5; 0106), and 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX; 1045) were from Tocris Bioscience. Poly-D-lysine hydrobromide (P1024) and other chemicals were from Sigma-Aldrich.

Luciferase assay. The full-length 3′ UTR of mouse MAP1B mRNA (NM_008634) was cloned at downstream of *Renilla* luciferase gene in the psiCHECK-2 vector (Promega), resulting in psiCHECK-2-MAP1B-

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Correspondence should be addressed to Che-Kun James Shen, Institute of Molecular Biology, Academia Sinica, 128 Academia Road, Section 2, Nankang, Taipei 115, Taiwan, Republic of China. E-mail: ckshen@imb.sinica.edu.tw.

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3′UTR. A mutant reporter psiCHECK-2-MAP1B-3′UTR-mt replacing the sequence UAGUUCUC in the 3′ UTR with UUCAAGAG was also constructed. Mouse neuroblastoma Neuro-2a cells were cotransfected with 0.1 μg of the reporter plasmid(s) plus 10 pmol of the synthetic miRNA duplexes (Sigma) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 48 h, the cells were lysed, and the luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Primary cultures and transfection. Primary hippocampal neuron cultures were prepared from embryonic day 17.5 C57BL/6JNarl mice of either sex. Cells were plated on ploy-D-lysine-coated dishes or coverslips at a density of 50,000 or 25,000 cells per square centimeter, respectively. The cultures were maintained in Neurobasal medium with B-27 supplement and GlutaMAX-1 (Invitrogen). On the days indicated, 100 nM synthetic siRNA or miRNA duplexes (Sigma) or anti-miRNA 2′-*O*-methyl oligonucleotides (Gene Link) were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Overexpression or inhibition of miR-146a-5p was confirmed by reverse transcription quantitative PCR (RT-qPCR) and fluorescence microscopy (data not shown).

RT-qPCR. Total RNA was extracted from neurons with TRIzol Reagent (Invitrogen). Reverse transcription of total RNAs and miRNAs was performed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems), respectively. qPCR analysis of mRNAs and miRNAs was performed with the TaqMan Gene Expression Assays (Applied Biosystems) and individual TaqMan MicroRNA Assays (Applied Biosystems), respectively.

Drug treatment. Primary cultured hippocampal neurons were treated with 10 μM CNQX (AMPAR antagonist) and 50 μM AP5 (NMDAR antagonist) for 5 min in the culture media. Subsequently, 100 μM DHPG was added to the media for 10 min. DHPG was washed out, and the neurons were further incubated in the media containing the antagonists for 10 min. Neurons were washed with HBSS (Invitrogen) before further experiments.

Immunofluorescence staining analysis. The analysis followed standard procedures with use of appropriate antibodies. For labeling of surface AMPAR subunit 1 (sGluA1), neurons were treated and labeled with the anti-GluA1 N terminus antibody as described previously (Volk et al., 2007). For labeling of total GluA1, MAP1B, and MAP2, neurons were fixed and permeabilized before adding the antibodies. The intensity of fluorescence was analyzed with ImageJ software, version 1.46 (NIH). All the primary and secondary dendrites of each clear neuron were included for measurement. Samples were collected from at least three biological replicates. Sample number (*n*) indicates the number of neurons analyzed.

Electrophysiology. Whole-cell patch clamp were performed to record mEPSCs. Neurons were incubated in extracellular solution, and patch electrodes were filled with intracellular solution as described previously (Snyder et al., 2001). The transfected neurons were voltage clamped at −70 mV (near the resting membrane potential of the cells), and the mEPSCs were recorded with the Axopatch 200B amplifier (Molecular Devices) and filtered at 1 kHz. The recordings were analyzed with Clampfit 10.2 (Molecular Devices). Only events with a monotonic rise time and exponential decay were included in the analysis. Samples were collected from more than three biological replicates.

Statistical analysis. All data are given as mean ± SEM unless indicated otherwise. Statistical significance between the means was calculated using paired Student's *t* test or using one-way ANOVA for comparison within groups.

Results

miR-146a-5p and miR-146b-5p target 3′ UTR of MAP1B mRNA

Using miRNA target prediction programs miRBase, microRNA.org, PITA, and miRDB (Griffiths-Jones et al., 2006; Kertesz et al., 2007; Betel et al., 2008; Wang, 2008), we identified putative miRNAs capable of binding to the MAP1B mRNA 3′ UTR. Furthermore, the expression of two dendritic miRNAs (Kye et al., 2007; Lugli et al., 2008), miR-146a-5p and miR-146b-5p, gradually increased during neuronal maturation (Fig. 1*A*), while the level of MAP1B protein decreased (Schoenfeld et al., 1989; Denny, 1991). To determine whether they could bind to the 3′ UTR of MAP1B mRNA and inhibit translation, miR-146a-5p or miR-146b-5p was cotransfected with a luciferase reporter containing the 3′ UTR of MAP1B. Both miRNAs, but not their mutant forms (miR-146a-5p-mt or miR-146b-5p-mt) with mutations in the seed region (nucleotides 2–8), reduced the luciferase activity by ~35% compared to the control (Fig. 1*B*). Moreover, the luciferase reporter carrying MAP1B-3′UTR with mutations of the recognition site of these two miRNAs failed to respond to the inhibitory effect of overexpression of either miR-146a-5p or miR-146b-5p (Fig. 1*C*). Together, these results suggested that both miR-146a-5p and miR-146b-5p targeted the MAP1B mRNA 3′ UTR and repressed translation.

miR-146a-5p is downregulated by group I mGluR activation in maturer neurons

We speculated that following presynaptic stimulation of receptors on postsynaptic densities, miR-146a-5p and miR-146b-5p might leave the 3′ UTR of MAP1B mRNA, thus triggering its translation. To test this, we examined the expression of miR-146a-5p and miR-146b-5p in mature neurons treated with DHPG, a group I mGluR agonist. Consistent with previous observations (Hou et al., 2006; Davidkova and Carroll, 2007), MAP1B protein was induced by DHPG in both DIV13 and DIV24 neurons (Fig. 1*D*), with no change in the level of mRNA (Fig. 1*E*). Although they shared an identical seed sequence and targeted the same mRNA 3′ UTR sequence (Fig. 1*B*), expression of miR-146a-5p, but not miR-146b-5p, in DIV24 neurons was significantly decreased upon DHPG treatment compared to the mock control (Fig. 1*E*). In parallel to the larger increase of miR-146a-5p in maturer neurons (Fig. 1*A*), the effects of DHPG on induction of MAP1B protein (*D*) and reduction of miR-146a-5p (*E*) were also greater in DIV24 neurons than DIV13 neurons. Therefore, elevation of the MAP1B protein upon activation of group I mGluR was mainly mediated on the translational level, and reduction of the level of miR-146a-5p was likely one of the causes in the maturer neurons.

miR-146a-5p regulates the amount of endogenous MAP1B protein

Next, the levels of both MAP1B protein and mRNA were examined upon overexpression or inhibition of miR-146a-5p in neurons. Overexpression of miR-146a-5p, but not its mutant form (miR-146a-5p-mt) or an unrelated miRNA, miR-106b-5p, reduced the amount of MAP1B protein by ~50% (Fig. 2*A*). Other synaptic proteins such as GluA1 and Limk1 were not affected by overexpression of miR-146a-5p, indicating the specificity of regulation (Fig. 2*B*). In contrast, overexpression of an anti-miR-146a-5p oligonucleotide blocking endogenous miR-146a-5p caused a significant increase in MAP1B protein (Fig. 2*C*). Some miRNAs could affect the stabilities of the target mRNAs in addition to repressing their translation (Wu and Belasco, 2005; Cohen et al., 2011). However, neither overexpression nor inhibition of miR-146a-5p changed the level of MAP1B mRNA (Fig. 2*D*,*E*). The data of Figures 1 and 2 suggested that miR-146a-5p regulated MAP1B mRNA translation through targeting of the 3′ UTR of MAP1B mRNA.

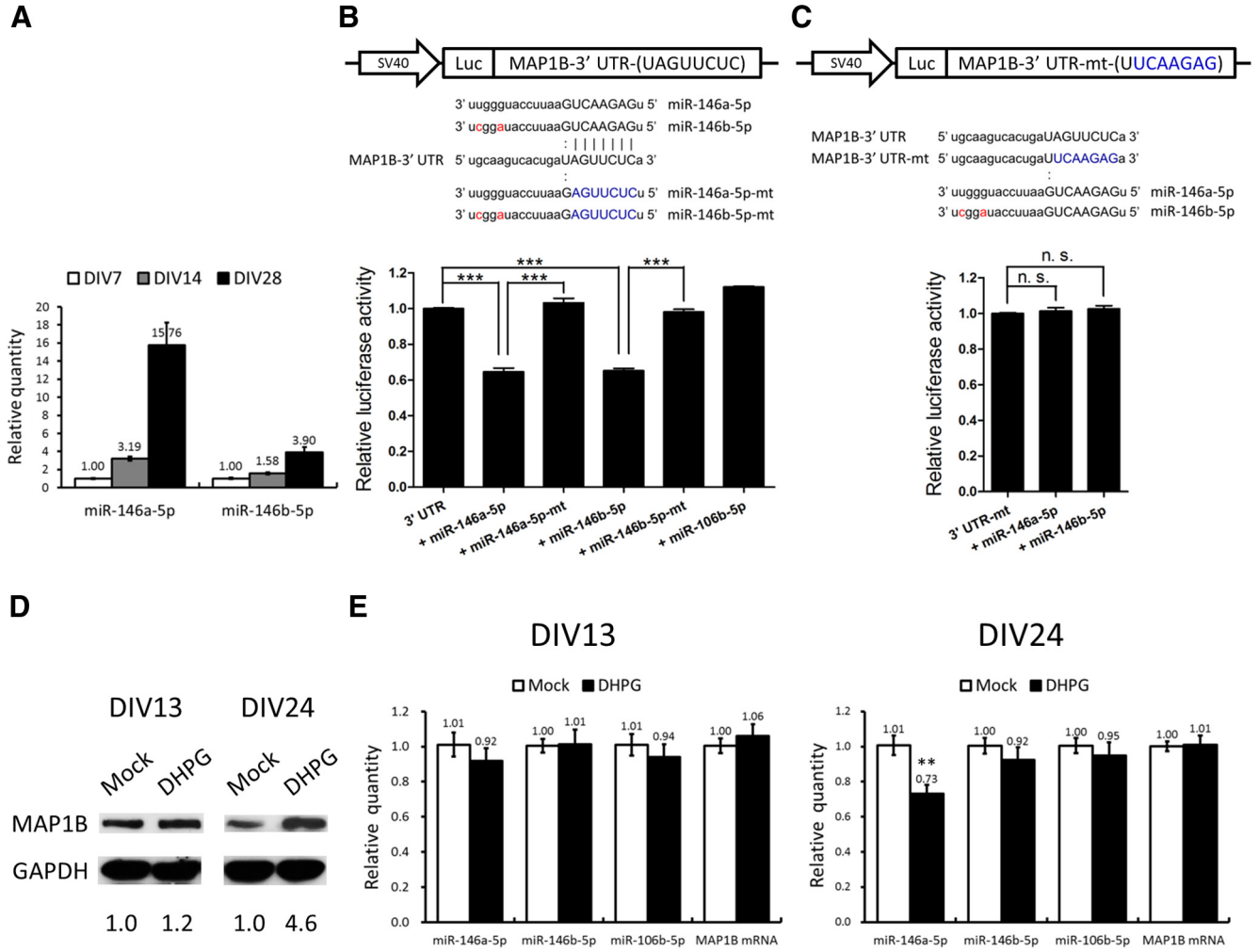

Figure 1. Correlation of MAP1B and miR-146a–5p. ***A***, The expression levels of miR-146a–5p and miR-146b–5p in DIV7, DIV14, and DIV28 neurons. The levels were determined by RT-qPCR. The amount of miRNA was first normalized to GAPDH and then quantified relative to the DIV7 samples. Note the larger increase of miR-146a–5p than miR-146b–5p during maturation of neurons in culture. Error bars represent the deviations of the mean ($n = 3$). ***B***, ***C***, Reporter assays of the luciferase reporter constructs psiCHECK-2-MAP1B-3′UTR (***B***) or psiCHECK-2-MAP1B-3′UTR-mt (***C***) in Neuro-2a cells with overexpressed wild-type or mutant (mt) miR-146a–5p or miR-146b–5p. Red letters indicate different nucleotides; blue letters denote mutated nucleotides in the mutant miRNAs or 3′ UTR of the reporter. Luciferase activities are relative to activity in Neuro-2a cells transfected with the wild-type reporter (***B***; $n = 6$, three biological repeats and duplicate assays) or the mutant reporter (***C***; $n = 12$, six biological repeats and duplicate assays). miR-106b–5p, which was not predicted to target the 3′ UTR of MAP1B, was used as the negative control. Error bars indicate SEM. $^{***}p < 0.001$ (one-way ANOVA with Tukey's test). n.s., Not significant. ***D***, ***E***, Effects of DHPG treatment on the expression levels of MAP1B and different RNAs in DIV13 or DIV24 neurons. The cultured neurons were treated with solvent control (mock) or 100 μM DHPG followed by Western blotting (***D***) or RT-qPCR (***E***), normalization to GAPDH, and quantification relative to mock control. In ***E***, $n = 6$ (three biological repeats and duplicate qPCR). Note that expression of miR-146a–5p, but not those of the other RNAs, was significantly changed by the DHPG treatment in DIV24 neurons. $^{**}p < 0.01$ (Student's *t* test).

Overexpression of miR-146a-5p attenuates group I mGluR-induced MAP1B protein synthesis and AMPAR endocytosis

Consistent with previous studies (Snyder et al., 2001; Davidkova and Carroll, 2007; Waung et al., 2008), we detected, by immunofluorescence staining, DHPG-induced increase of the dendritic MAP1B protein and reduction of sGluA1 (Fig. 3*A*). Furthermore, either MAP1B siRNA- or miR-146a-5p-mediated MAP1B knockdown reduced the level of dendritic MAP1B protein and did not affect the basal level of sGluA1 (Fig. 3*B*). However, in contrast to the control miR-106b-5p, overexpression of miR-146a-5p counteracted the DHPG-induced elevation of MAP1B protein, thus resulting in blockage of the DHPG-induced AMPAR endocytosis (Fig. 3*B*). Additionally, inhibition of miR-146a-5p did not significantly further enhance the effects of DHPG on MAP1B protein synthesis and AMAPR endocytosis (data not shown). Together, these data suggested that miR-146a-5p was a, but not the, major downstream effector of group I mGluR signaling.

Inhibition of miR-146a-5p increases dendritic MAP1B protein, causing AMPAR endocytosis and long-term depression

The data in Figures 2 and 3 suggested that upregulation of the MAP1B protein expression by a blockade of miR-146a-5p in mature neurons should lead to AMPAR internalization. As expected, an increase (47%) of the dendritic MAP1B protein and a concomitant decrease of surface AMPARs (47%) were observed in anti-miR-146a-5p-transfected neurons compared to those transfected with a scrambled control oligonucleotide (Fig. 4*A*). However, the intensities of the total GluA1 (surface and internalized ones) were similar in neurons transfected with anti-miR-146a-5p and scrambled control oligonucleotide, respectively (Fig. 4*B*), ruling out the contribution of inhibition of GluA1 expression on the decrease of the surface AMPARs in anti-miR-146a-5p-transfected neurons.

A role of miR-146a-5p in the regulation of AMPAR-mediated mEPSCs was also revealed (Fig. 4*C*). In comparison to the scrambled control, anti-miR-146a-5p oligonucleotide transfection significantly reduced the mEPSC frequency (for at least 30 min;

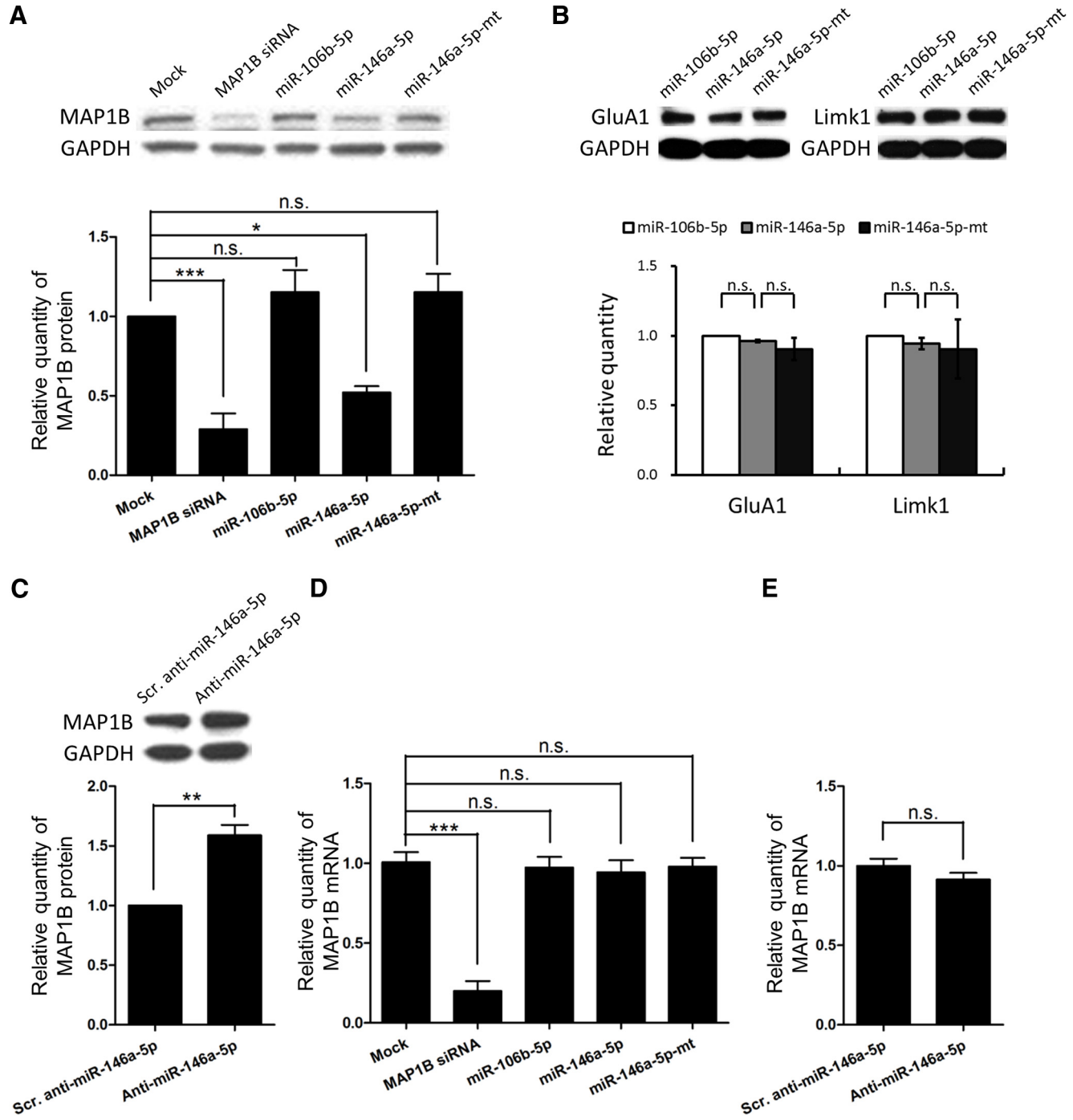

Figure 2. Modulation of MAP1B protein by miR-146a–5p. ***A–E***, The amounts of synaptic proteins (***A–C***) and MAP1B mRNA (***D***, ***E***) were determined by Western blotting and RT-qPCR, respectively. ***A***, ***B***, ***D***, Analysis of neurons transfected with different miRNAs at DIV9 and harvested at DIV13. MAP1B siRNA transfection was used as the positive control. The levels of proteins or mRNAs were normalized to GAPDH, and they are relative to the mock transfected control (***A***, ***D***) or miR-106b–5p transfection (***B***). ***C***, ***E***, Analysis of neurons transfected with anti-miRNAs at DIV13 for 4 h. The levels were normalized to GAPDH, and they are relative to that in neurons transfected with the scrambled anti-miR-146a–5p. In ***A***, $n = 4$ (four biological repeats); in ***B***, $n = 3$ (three biological repeats); in ***C***, $n = 5$ (five biological repeats). ***D***, ***E***, $n = 6$ (three biological repeats and duplicate qPCR). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (***A***, ***B***, ***D***, one-way ANOVA with Tukey's test; ***C***, ***E***, Student's *t* test). n.s., Not significant.

data not shown) but did not affect amplitude (Fig. 4*C*). The reduction in frequency of mEPSCs reflected a reduction in the number of AMPAR-containing synapses (Carroll et al., 1999), and thus was consistent with the induction of AMPAR endocytosis by inhibition of miR-146a-5p (Fig. 4*A*). Furthermore, MAP1B knockdown by MAP1B siRNA, but not a control siRNA, rescued the decreased mEPSC frequency caused by anti-miR-146a-5p (Fig. 4*C*), suggesting that MAP1B is a downstream effector of miR-146a-5p in the regulation of AMAPR-mediated synaptic transmission. Therefore, miR-146a-5p played an essential role in the maintenance of synaptic activity of mature neurons.

Discussion

Gene expression is fine-tuned by miRNAs through several mechanisms, including translational inhibition. We show in this study that

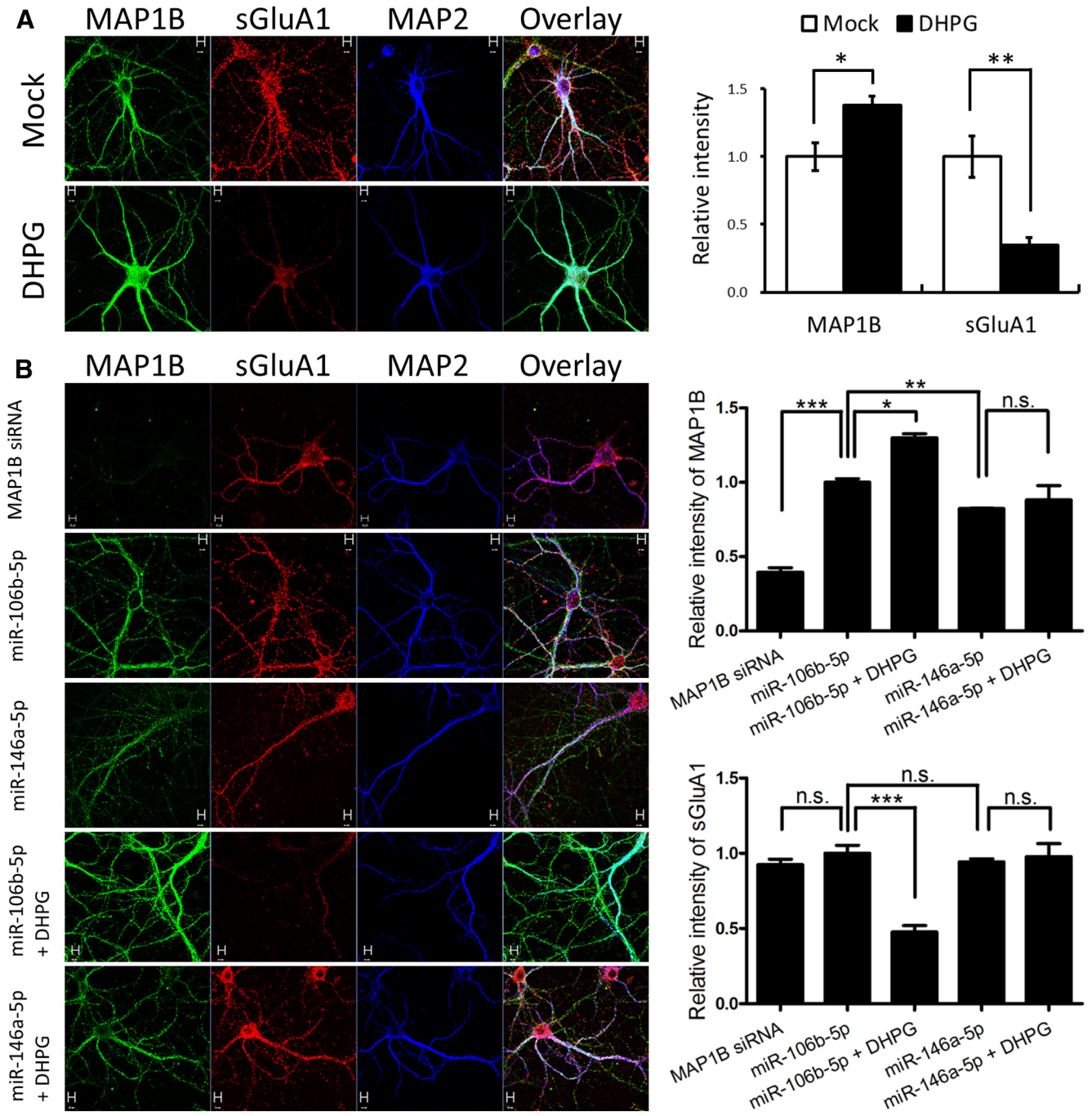

Figure 3. Effects of overexpression of miR-146a–5p on DHPG-induced upregulation of MAP1B protein and AMPAR internalization. ***A***, Immunofluorescence staining analysis of DIV11 neurons treated with solvent control (mock) or 100 μM DHPG. Neurons were stained for MAP1B (green), sGluA1 (red), and MAP2 (blue). Histogram shows quantification of the mean intensities of MAP1B and sGluA1 of the DHPG-treated neurons ($n = 9$) relative to the mock neurons ($n = 5$). ***B***, Immunofluorescence staining analysis of DIV9 neurons transfected with MAP1B siRNA, control miRNA (miR-106b–5p), or miR146a–5p for 2 d, and then treated without or with 100 μM DHPG. Dendritic MAP1B (green), sGluA1 (red), and MAP2 (blue) were then analyzed. Histograms show the intensities of MAP1B and sGluA1, respectively, of the neurons treated with MAP1B siRNA ($n = 12$), miR-106b–5p + DHPG ($n = 11$), miR-146a–5p ($n = 13$), or miR-146a–5p + DHPG ($n = 11$) relative to the miR-106b–5p-transfected neurons ($n = 6$). Scale bars: 10 μm. Error bars indicate SEM. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (***A***, Student's *t* test; ***B***, one-way ANOVA with Tukey's test). n.s., Not significant.

the miRNA miR-146a-5p is involved in the translational repression of MAP1B, a protein facilitating group I mGluR-dependent endocytosis of AMPARs and consequently the decline in synaptic transmission. miR-146a-5p maintains a basal level of MAP1B translation through binding to the 3′ UTR of its mRNA. Activation of group I mGluR by agonists, such as DHPG, relieves the translational repression, giving rise to MAP1B upregulation followed by AMPAR internalization and long-term depression.

Consistent with a model in which miRNAs imperfectly pair to the 3′ UTR of target mRNAs to repress translation (Filipowicz et al., 2008; Fabian et al., 2010), we found that miR-146a-5p targets the 3′ UTR of MAP1B mRNA through its seed region required for the recognition and translational inhibition of the mRNA (Fig. 1*B*,*C*). Furthermore, modulation of miR-146a-5p affects the amount of MAP1B protein, but not mRNA (Fig. 2). In addition, overexpression of miR-146a-5p diminishes the DHPG-induced AMPAR endocytosis (Fig. 3), while acute blockade by an

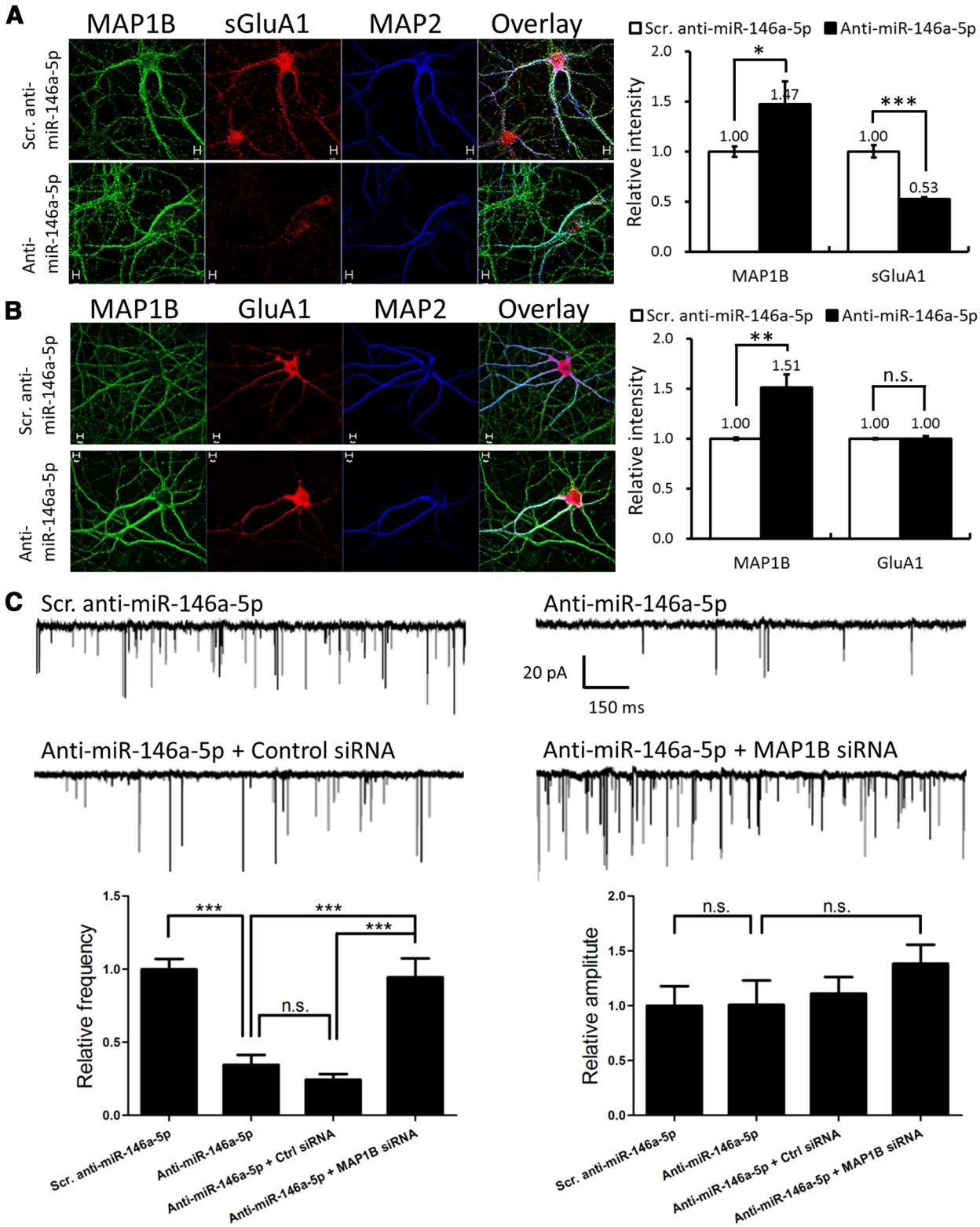

Figure 4. Effects of inhibition of miR-146a–5p on AMPAR endocytosis and synaptic activity. ***A***, ***B***, Immunofluorescence staining analysis of DIV11 (***A***) and DIV13 (***B***) neurons transfected with scrambled control oligonucleotide or anti-miR-146a–5p oligonucleotide for 4 h. The levels of dendritic MAP1B (green), sGluA1 (red; ***A***), GluA1 (red; ***B***), and MAP2 (blue) were then analyzed. Scale bars: 10 μm. Histograms show the levels of MAP1B, sGluA1, and GluA1 of the transfected neurons compared to the scrambled (Scr.) oligonucleotide-transfected neurons. Scr. anti-miR-146a–5p, $n = 7$ (***A***, ***B***); anti-miR-146a–5p, $n = 5$ (***A***) and $n = 7$ (***B***). ***C***, mEPSC measurements of DIV11 neurons transfected with scrambled control oligonucleotide or anti-miR-146a–5p oligonucleotide for 4 h, and DIV10 neurons cotransfected with anti-miR-146a–5p and MAP1B siRNA or control siRNA for 1 d. The representative traces of mEPSCs are shown on the top, and the quantitative analysis of the mEPSC frequency and the mEPSC amplitude is shown in the bottom two histograms. The mean frequencies or amplitudes are relative to the scrambled oligonucleotide-transfected neurons. Scr. anti-miR-146a–5p, $n = 5$; anti-miR-146a–5p, $n = 7$; anti-miR-146a–5p + MAP1B siRNA, $n = 5$; anti-miR-146a–5p + Control siRNA, $n = 7$. Error bars indicate SEM. $^{*}p < 0.05$, $^{**}p < 0.01$; $^{***}p < 0.001$ (***A***, ***B***, Student's *t* test; ***C***, one-way ANOVA with Tukey's test). n.s., Not significant.

anti-miR-146a-5p oligonucleotide leads to an increase in dendritic MAP1B protein (Fig. 4*A*), facilitation of AMPAR internalization (Fig. 4*A*), and reduction in the frequency of AMPAR-mediated mEPSCs (Fig. 4*C*). Moreover, the anti-miR-146a-5p-induced reduction of mEPSC frequency is rescued by MAP1B siRNA (Fig. 4*C*). Collectively, our results suggest that miR-146a-5p regulates mGluR-LTD in part through MAP1B.

Similarly to miR-146a-5p, another miRNA miR-9 has been found to locally target MAP1B and interfere with its translation in distal axons of early developing cortical neurons (Dajas-Bailador et al., 2012). Thus, MAP1B coordinates different functions during neuronal development and in mature dendrites. In early developing neurons, MAP1B is highly expressed in the growing axons, where it controls axonal growth (Gonzalez-Billault et al., 2001; Bouquet et al., 2004) and is regulated by miR-9 (Dajas-Bailador et al., 2012). In mature neurons, MAP1B migrates into the postsynaptic densities where it modulates group I mGluR-dependent endocytosis of AMPARs (Davidkova and Carroll, 2007), and its translation is regulated by miR-146a-5p (present study). Whether miR-9 and miR-146a-5p work coordinately in mature dendrites remains to be examined.

It is likely that more than one pathway regulates the relief of translational repression of MAP1B by miR-146a-5p upon stimulation of group I mGluR, e.g., by DHPG. Among the possibilities is the activity-induced degradation of miRNP proteins leading to a relief of mRNAs from silencing by miRNAs (Banerjee et al., 2009). Furthermore, since there is only little to 30% decrease in the level of miR-146a-5p upon DHPG treatment (Fig. 1*E*), the reduction of miR-146a-5p level is likely not the major factor causing the induction of MAP1B translation. However, the level of primary transcript of miR-146a-5p in DIV24 neurons is not changed by DHPG treatment (data not shown), and stimulation has been shown to accelerate the turnover of neuronal miRNAs (Krol et al., 2010). Thus, DHPG-induced degradation of miR-146a-5p could play a role, albeit not a major one, in the upregulation of MAP1B protein in the maturer neurons. Notably, upon treatment of the neurons with DHPG, the MAP1B mRNA is transported from the soma to the dendrites and translation of this mRNA is upregulated (Hou et al., 2006; Davidkova and Carroll, 2007; Dictenberg et al., 2008). These findings together with our data suggest that under normal conditions, miR-146a-5p might also be localized in the soma, as the MAP1B mRNA, to repress translation of MAP1B.

Misregulation of the expression of miR-146a-5p could be involved in the pathogenesis of several neurological disorders. Interestingly, the majority of cases of sporadic RTT are caused by mutations in the methyl-CpG-binding protein 2 (MECP2) (Neul and Zoghbi, 2004), and, significantly, miR-146a-5p expression is downregulated in a MECP2-null mouse model of this disease (Urdinguio et al., 2010). This suggests that miR-146a-5p transcription may be regulated by DNA methylation. Moreover, a decrease in the frequency of mEPSCs, an effect of inhibition of miR-146a-5p (Fig. 4*C*) has been observed in MECP2-deficient neurons (Nelson et al., 2006). Our data, together with these studies, suggest that pathogenesis of RTT could result in part from downregulation of miR-146a-5p that leads to impaired synaptic transmission of the diseased neurons due to increased MAP1B translation and consequent AMPAR endocytosis.

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