Dynamic Association of the Fragile X Mental Retardation Protein as a Messenger Ribonucleoprotein between Microtubules and Polyribosomes

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The fragile X mental retardation protein (FMRP) is a selective RNA-binding protein that regulates translation and plays essential roles in synaptic function. FMRP is bound to specific mRNA ligands, actively transported into neuronal processes in a microtubule-dependent manner, and associated with polyribosomes engaged in translation elongation. However, the biochemical relationship between FMRP–microtubule association and FMRP–polyribosome association remains elusive. Here, we report that although the majority of FMRP is incorporated into elongating polyribosomes in the soluble cytoplasm, microtubule-associated FMRP is predominantly retained in translationally dormant, polyribosome-free messenger ribonucleoprotein (mRNP) complexes. Interestingly, FMRP–microtubule association is increased when mRNPs are dynamically released from polyribosomes as a result of inhibiting translation initiation. Furthermore, the I304N mutant FMRP that fails to be incorporated into polyribosomes is associated with microtubules in mRNP particles and transported into neuronal dendrites in a microtubule-dependent, 3,5-dihydroxyphenylglycine-stimulated manner with similar kinetics to that of wild-type FMRP. Hence, polyribosome-free FMRP–mRNP complexes travel on microtubules and wait for activity-dependent translational derepression at the site of function. The dual participation of FMRP in dormant mRNPs and polyribosomes suggests distinct roles of FMRP in dendritic transport and translational regulation, two distinct phases that control local protein production to accommodate synaptic plasticity.

INTRODUCTION

The fragile X mental retardation protein (FMRP) is a critical factor for neuronal function and development. The lack of FMRP causes fragile X syndrome, the most frequent form of familial mental retardation in humans (O’Donnell and Warren, 2002; Bear et al., 2004; Bagli and Greenough, 2005; Penagarikano et al., 2007). FMRP is a selective RNA-binding protein abundantly expressed in brain neurons, where it is incorporated into polyribosomes that are already engaged in translation elongation (Feng et al., 1997a; Ceman et al., 2003; Stefani et al., 2004), and it is implicated in controlling translation efficiency of its mRNA ligands with various potential mechanisms (Zalfa et al., 2003; Feng, 2002; Antar and Bassell, 2003; Jin and Warren, 2003; Garber et al., 2006). Consistent with this view, FMRP has been shown to suppress translation of its bound mRNAs in vitro (Laggerbauer et al., 2001; Li et al., 2001), in transfected cells (Mazroui et al., 2002; Wang et al., 2004), and in the brain (Lu et al., 2004; Qin et al., 2005). In addition, FMRP and its mRNA ligands are transported to dendritic shafts and spines as well as developing growth cones (Miyashiro et al., 2003; Antar et al., 2004, 2005; Ferrari et al., 2007), providing a mechanism for FMRP to regulate local translation to accommodate synaptic development and plasticity (Bagli and Greenough, 2005). Indeed, dendritic transport of FMRP and Fmr1 mRNA, as well as localized translation of FMRP at the synaptic terminals, can be stimulated by the activation of group I metabotropic glutamate receptors (GP1-mGluRs) (Weiler et al., 1997; Antar et al., 2004; Ferrari et al., 2007). Moreover, the absence of FMRP results in deregulation of GP1-mGluR–stimulated protein synthesis in synaptoneurosomes (Weiler et al., 2004; Mudde et al., 2007). Hence, the absence of FMRP-regulated local translation in response to synaptic activity is proposed as an underlying mechanism for the mental impairment in fragile X syndrome (Willemsen et al., 2004; Penagarikano et al., 2007).

Several microscopic studies indicate that FMRP is transported into neuronal processes in a microtubule-dependent manner (De Diego Otero et al., 2002; Antar et al., 2005). However, despite the fact that FMRP colocalizes with ribosome proteins and microtubule motors (De Diego Otero et al., 2002; Ling et al., 2004) and can be detected in biochemically isolated large granules containing clustered ribosomes (Aschrafi et al., 2005; Elvira et al., 2006), the biochemical relationship between FMRP-associated polyribosomes and microtubule-dependent FMRP transport remains undefined. It is not understood whether FMRP travels on microtubules after it is incorporated into polyribosomes through transla-
tion initiation on its mRNA targets, or alternatively whether dendritic transport of FMRP is in the form of polyribosome-free messenger ribonucleoprotein (mRNP) complexes that are sequen
ted from translation initiation. How FMRP con-
trols transport and translation of its mRNA targets, which in
turn governs neuronal function and development, is an
important question in understanding FMRP function and pathogenesis of fragile X mental retardation.

The goal of this study is to dissect the biochemical relationship between the dynamic association of FMRP with polyribosomes and microtubules. We found that in contrast to the microtubule-dependent dendritic transport of FMRP (De Diego Otero et al., 2002; Antar et al., 2005), polyribosome association of FMRP is not affected by microtubule disruption. In fact, microtubule-bound FMRP is not associated with polyribosomes, but it largely cosediments with mRNP complexes and ribosomal subunits on a linear sucrose gra-
dient. Releasing FMRP into polyribosome-free mRNP com-
plexes, but not short polyribosomes, as a result of pharma-
cological inhibition of translation, increased the amount of microtubule-associated FMRP. Moreover, the I304N mutant
FMRP that fails to associate with polyribosomes (Feng et al.,
1997a) binds microtubules, colocalizes with RNA on micro-
tubule polymers, and is transported into the dendrites of
hippocampal neurons in a microtubule-dependent manner
with similar kinetics as that of wild-type FMRP. Together,
these results suggest that microtubule-dependent transport of FMRP and its mRNA ligands is largely in the form of translocationally dormant free mRNP complexes, waiting to be activated for polyribosome association and local translation upon synaptic stimulation.

MATERIALS AND METHODS

Cell Cultures

The CAD cell line was propagated and maintained at 60 – 85% confluence in DMEM/F-12 containing 8 – 10% fetal bovine serum (FBS) (Invitrogen, Carls-
bad, CA) as described previously (Qi et al., 1997). The immortalized fragil X fibroblast cell line was maintained at 60 – 85% confluence in DMEM contain-
ing 10% FBS. Transfection of the fragile X fibroblast cell line was performed using the Nucleofector technology following manufacturer’s protocol (Ampato Biosystems, Gaithersburg, MD). Hippocampal neurons from day 18 rat em-
broys or from P0 FVB mice were cultured on glass coverslips (18 mm for
immunofluorescence and 40 mm for live imaging; Bioptechs, Butler, PA) and
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bryos or from P0 FVB mice were cultured on glass coverslips (18 mm for
immunofluorescence and 40 mm for live imaging; Bioptechs, Butler, PA) and
b
RESULTS

**FMRP–Polyribosome Association Is Independent of Microtubules**

Several lines of evidence indicate that microtubules are essential for dendritic transport of FMRP (De Diego Otero et al., 2002; Antar et al., 2005). However, whether FMRP–polyribosome association also depends on microtubule integrity has not been studied previously. To address this question, we analyzed FMRP-polyribosome association in the immortalized brain catecholaminergic neuronal cell line CAD using linear sucrose gradient fractionation. As shown in Figure 1A, the majority of cytoplasmic FMRP (~80%) cofractionated with large polyribosome complexes in cytoplasmic extracts (fractions 6–11). EDTA treatment resulted in dissociation of polyribosomes into subunits (Figure 1B, fractions 1–5). Interestingly, when microtubules are either robustly bundled by paclitaxel-mediated stabilization (Figure 1C) or severely depolymerized by nocodazole treatment (Figure 1D), FMRP-polyribosome association was unaffected. A nearly identical sedimentation profile of FMRP was observed in untreated cells (Figure 1A) and in cells with severe microtubule perturbation (Figure 1, C and D). In addition, disruption of actin dynamics by cytochalasin B treatment did not affect FMRP–polyribosome association (data not shown). Hence, unlike dendritic transport of FMRP, polyribosome association of FMRP is independent of microtubule and actin cytoskeletal integrity.

**FMRP–Microtubule Association Is Sensitive to RNAse Treatment, Predominantly in the Form of Polyribosome-free mRNP Complexes**

To determine how much cytoplasmic FMRP is associated with the microtubule cytoskeleton, we isolated soluble cytoplasm and cytoskeletal pellet from the postnuclear extracts derived from CAD cells that were mock treated or exposed to drugs that alter assembly of either the microtubule or the actin cytoskeleton (Figure 2). Immunoblot analysis revealed that ~10% of total FMRP was associated with the cytoskeleton pellet (P), whereas the majority of FMRP was present in the postcytoskeletal supernatant (S). Increased microtubule assembly, as a result of paclitaxel pretreatment, doubled the amount of FMRP associated with the cytoskeletal pellet (Figure 2A). In addition, RNAse treatment of the paclitaxel-stabilized microtubule pellet significantly reduced the amount of FMRP detected by immunoblot (Figure 2B), suggesting that microtubule-associated FMRP requires RNA. Reciprocally, destabilization of microtubules by nocodazole reduced the amount of FMRP in the cytoskeletal pellet (Figure 2C). In contrast, disruption of the actin cytoskeleton by pretreatment with cytochalasin B did not alter the amount of cytoskeleton-associated FMRP (Figure 2D). These results suggest that FMRP-containing RNA complexes associate with microtubules but not with actin polymers in total cytoskeleton.

To delineate the biochemical nature of the microtubule-associated FMRP-containing complexes, we performed parallel linear sucrose gradient fractionation experiments using extracts derived from the microtubule pellet (MT) and that...
The I304N mutation does not affect FMRP–microtubule association, despite the inability for incorporation into polyribosomes

The I304N mutation in the second KH domain of FMRP was identified from a patient with extremely severe clinical expression of fragile X syndrome (De Boulle et al., 1993). This mutation ablates FMRP–polyribosome association (Feng et al., 1997a) and affects FMRP binding to the kissing complex RNA ligand (Darnell et al., 2005a), but it does not affect binding to the G-quartet FMRP-binding RNA motif (Darnell et al., 2001) and cellular poly(A) RNA (Feng et al., 1997a). To test whether the I304N mutation may affect FMRP–microtubule association, we transfected fluorescent-tagged wild-type and I304N mutant FMRP individually into an immortalized fibroblast cell line derived from a fragile X patient that lacks endogenous FMRP (Figure 5). Confocal microscopy revealed that both the wild-type and the I304N mutant FMRP formed particles that were clearly aligned on individual microtubules in the cell body and at the leading edge (Figure 5A). In addition, in the transfected fragile X cells, comparable levels of the wild-type and I304N-FMRP were detected in the paclitaxel-stabilized microtubule pellets (Figure 5B), suggesting that I304N-FMRP and wild-type FMRP associates with microtubules with similar efficiency, despite the fact that the I304N mutation is incapable for polyribosome association (Feng et al., 1997a).

The I304N mutant FMRP is transported to neuronal dendrites in a microtubule-dependent manner, which is stimulated upon activation of GP1-mGluR

To examine whether I304N-FMRP can form RNP complexes on microtubules in primary neurons, the GFP-I304N-FMRP expression construct was microinjected into cortical neurons. Similar to wild-type FMRP (Pang et al., 1998), I304N-FMRP formed punctate particles that were clearly aligned on individual microtubules in the dendritic tree, suggesting that the I304N mutation does not affect the interaction of FMRP with microtubules in living cells.
fusion construct was transfected into hippocampal cultures (10 DIV) and processed for immunofluorescence analysis (Figure 5C). The GFP-I304N-FMRP fusion protein clearly formed granules typical for mRNPs. However, these complexes seemed to be somewhat diffuse in dendrites, with apparently fewer dendritic particles and smaller sizes than those seen in neurons stained for endogenous FMRP and the wild-type FMRP-GFP fusion protein (Antar et al., 2004). Nonetheless, the GFP-I304N-FMRP granules (green) contained RNA as marked by propidium iodide (red), and they were aligned on individual microtubule bundles (blue). Furthermore, time-lapse recording revealed active movement of these complexes into dendrites (see below; also see Supplemental Movies). When transfected neurons were treated with the GP1-mGlur agonist DHPG, we observed a significant increase in the dendritic levels of the GFP-I304N-FMRP compared with that in mock-treated cells (Figure 6, A–D). Quantitative analysis revealed an approximately twofold increase of dendritic localization in response to DHPG treatment (Figure 6E), similar to the DHPG-stimulated localization reported previously for the GFP-tagged wild-type FMRP (Antar et al., 2004).

To further determine whether the GFP-I304N-FMRP is transported into dendrites in a microtubule-dependent manner, we performed FRAP in live neurons. Transfected hippocampal neurons were stimulated with DHPG, and the transport kinetics of the I304N-FMRP was measured in the absence and presence of nocodazole (Figure 7). A robust transport of the I304N-FMRP into dendrites was observed after photobleaching (Figure 7, B–D). In contrast, a brief treatment (30–60 min) by nocodazole markedly impaired the fluorescence recovery after photobleaching (Figure 7, F–H). Quantification of recovery rates (Figure 7I) revealed that nocodazole treatment caused a 32-fold decrease in transport kinetics for the I304N-FMRP, similar to that for wild-type FMRP (Antar et al., 2005). Together, these data suggest that the I304N mutation does not affect the ability of FMRP to form transport-competent mRNP complexes or binding microtubules in neurons, despite the fact that this mutation prevents FMRP from association with polyribosomes (Feng et al., 1997a), dimerization (Laggerbauer et al., 2001), and binding to specific RNA ligands (Darnell et al., 2005a).

**DISCUSSION**

In this study, we show for the first time that FMRP is associated with microtubules predominantly as translationally dormant, polyribosome-free mRNP. In contrast, the majority of cellular FMRP is incorporated into polyribosomes in the soluble cytoplasm, independently of microtubule integrity. FMRP–polyribosome association is neither required for microtubule association nor for dendritic transport of FMRP. Hence, neuronal FMRP are subjected to the following alternative cellular pathways: 1) to be incorporated into polyribosomes and govern translation elongation efficiency of its bound mRNA ligands, but perhaps lose the ability to associate with microtubules; or 2) to be sequestered from translation initiation and travel on microtubules toward neuronal dendrites. On arrival at the functional destination, these FMRP–mRNP complexes will be subjected to activity-dependent translational derepression, which controls localized production of critical proteins that are required for synaptic plasticity.

FMRP–polyribosome association is observed in various cell lines and in the mouse brain (Eberhart et al., 1996; Corbin et al., 1997; Ceman et al., 2003; Khandjian et al., 2004; Stefani et al., 2004; Wang et al., 2004). The FMRP-containing polyribosomes are engaged in translation elongation, but they display delayed ribosome runoff (Feng et al., 1997a; Ceman et al., 2003). Hence, suppression of translation elongation is one of the proposed models for FMRP to inhibit protein synthesis, which may be derepressed upon serine-phosphorylation of FMRP (Ceman et al., 2003). In addition to its presence in polyribosomes, FMRP is also known to associate with microtubules and motor proteins (De Diego Otero et al., 2002; Kanai et al., 2004) to be transported into neuronal processes in a microtubule-dependent manner (Antar et al., 2005). Unlike dendritic transport of FMRP, polyribosome-association of FMRP is not affected by microtubule disruption (Figure 1). Nonetheless, whether and how FMRP–polyribosome...
some association is related to FMRP–microtubule association and dendritic transport of FMRP was not addressed by previous studies.

Association of mRNA and polyribosomes with microtubules has been well documented (Bassell et al., 1994; Hamill et al., 1994). In addition, a number of RNA-binding proteins, including FMRP, are capable of association with both ribosomes and microtubules and can be detected in neuronal processes (Bolognani et al., 2004; Brendel et al., 2004; Huang and Richter, 2004; Huttelmaier et al., 2005; Kosturko et al., 2005). A major paradigm for dendritic mRNA transport is the presence of translocating mRNAs in large granules that contain various RNA-binding proteins and densely packed ribosome clusters arrested from translation (for review, see Krichevsky and Kosik, 2001; Kiebler and Bassell, 2006). Although FMRP was detected in ribosome-containing large granules isolated from mouse brain (Kanai et al., 2004; Aschrafi et al., 2005; Elvira et al., 2006), how much FMRP is present in these granules and whether these granules are in the process to be transported into dendrites are not determined. Moreover, no evidence indicates that these clustered ribosomes are polyribosomes engaged in translation elongation. In fact, a recent proteomic study by Elvira et al. (2006) suggests that these large granules contain “an amorphous collection of ribosomes” unlikely representing polyribosomes. Because these large granules are not stimulated for dendritic localization by neuronal activation, whereas dendritic transport of FMRP is vigorously stimulated by neuronal depolarization and activation of mGluRs (Antar et al., 2004), FMRP must be present in other types of RNA-containing granules (Kiebler and Bassell, 2006).

Indeed, several independent lines of evidence indicate that specific RNA-binding proteins that inhibit translation initiation also facilitate dendritic localization (Huang et al., 2003; Huttelmaier et al., 2005). This argues that some mRNPs must be transported in the form of dormant particles sequestered from polyribosomes (Huang and Richter, 2004; Dahm and Kiebler, 2005). However, it is not clear how this model pertains with the ribosome clusters in the large granules detected in the neuronal dendrites. Moreover, direct evidence for this model is not provided previously. Our results clearly show that in the CAD neuronal cell line, FMRP-containing complexes isolated from microtubules cofractionate with free mRNPs and ribosomal subunits (Figure 3), but not with large polyribosomes. In addition, these microtu-

![Figure 4](image-url)
bule-associated FMRP complexes contain RNA (Figure 5C), and they can be removed from microtubules by RNase treatment (Figure 2B). Furthermore, releasing FMRP from polyribosomes into mRNPs by inhibiting translation initiation increased the FMRP–microtubule association (Figure 4A). In contrast, releasing FMRP from large polyribosomes

Figure 5. The I304N mutation in FMRP that abrogates FMRP–polyribosome association does not affect the association of FMRP with microtubules. (A) An immortalized fragile X fibroblast cell line was transfected to express RFP-FMRP (a) or GFP-I304N-FMRP (b) for 20 h. Cells were stained for α-tubulin to visualize individual microtubule polymers. Enlarged image indicating the alignment of RFP-FMRP and GFP-I304N granules (white rectangles in a and b) with individual microtubule bundles are shown in c and d. (B) Immunoblot analysis of paclitaxel-stabilized MT isolated from post-nuclear lysates (Inp) from a fragile X fibroblast cell line expressing Flag-tagged wild-type FMRP or Flag-I304N FMRP. The same blot was reprobed by antibodies for eIF5a and total tubulin (Tub). (C) Colocalization of the GFP-I304N-FMRP with RNA and microtubules in the dendrites. Primary rat hippocampal neurons (10 DIV) were transfected with GFP-FMRP-I304N for 8 h before fixed and stained for total RNA and tubulin. Top panels show transfected neuron expressing the GFP-I304N-FMRP, in which RNA granules stained by propidium iodide and tubulin polymers stained by Cy5 fluorescence of anti-tubulin antibodies are visualized by fluorescent microscopy. A proximal dendritic segment of a transfected neuron (white outlined) is shown with higher magnification in the bottom panels. Arrows highlight areas where FMRP-I304N granules (green) colocalize with RNA (red) on microtubule bundles (blue) within the dendrite.

Figure 6. DHPG stimulates dendritic transport of FMRP-I304N in hippocampal neurons. Primary hippocampal neurons (10 DIV) derived from P0 FVB mice were transfected with GFP-I304N-FMRP for 12–16 h before subjected to DHPG treatment. (A and B) Representative images of GFP-I304N-FMRP granules in an unstimulated control cell and a DHPG-stimulated cell. (C and D) Immunofluorescent staining of MAP2 marks the dendrites in the corresponding cells in A and B. (E) Average ratios for dendrite/cell body fluorescence in DHPG-stimulated (hatched bar) and unstimulated cells (white bar) were calculated using at least 34 dendrites from more than 12 cells within three separate experiments are expressed graphically. *p < 0.01 as determined by Student’s t test. Bar 20 μm (A).
Because many of the large granules are immobile in den-
mRNP complexes after they left the soma remains unknown.
The mechanism by which large polyribosomes are sequestered from translation, some may
be a format for dendritic transport, or alternatively as a local storage compartment for mRNAs under transla-
tional arrest and are poised for release for active translation in dendrites (Krichevsky and Kosik, 2001), still remains as an
interesting question to be addressed by future studies.

Consistent with the results that the microtubule-associated FMRP–mRNP complexes are not incorporated into
elongating polyribosomes, the I304N mutation that abolishes FMRP–polyribosome association (Feng et al., 1997a)
do not affect the efficiency of FMRP–microtubule association (Figure 5) or dendritic transport of FMRP (Figures 6 and
7). This is in agreement with a previous study showing microtubule-dependent localization of GFP-I304N-FMRP in
the neurites of PC12 cells (Schrier et al., 2004). However, this previous report could not detect I304N-FMRP in visible
RNA-containing granules to resolve the incorporation of I304N-FMRP into RNP's. With our high-resolution imaging
approach, the GFP-I304N-FMRP can be clearly visualized in granules, colocalized with RNA on microtubules in hip-
pocampal neurons (Figure 6C), and actively transported into dendrites (Figure 7). Although the I304N mutation partially
abrogates the activity of FMRP in binding a subclass of mRNAs, represented by the kissing complex (Darnell et al.,
2005a), it does not prevent FMRP from binding the G-quartet and poly(A) RNA (Feng et al., 1997a; Darnell et al., 2001).
Interestingly, the GFP-I304N-FMRP granules are smaller than those formed by the wild-type GFP-FMRP (Antar et al.,
2004), possibly due to the oligomerization defects caused by the I304N mutation (Laggerbauer et al., 2001). The inability
for I304N to associate with ribosomes may also contribute to the small size of these granules. Nonetheless, quantitative
FRAP analysis demonstrated microtubule-dependent dendritic transport of I304N-FMRP granules (Figure 7), which is
stimulated upon activation of GPl-mGluR (Figure 6) with similar kinetics of transport as that for the wild-type FMRP
(Antar et al., 2005). These results provide a parallel line of evidence suggesting that dendritic transport of FMRP is
independent and can be uncoupled from its association with polyribosomes.

Our result that FMRP can be detected in translationally repressed particles despite its predominant polyribosome
association (Figure 3) is consistent with previous reports (Mazroui et al., 2002; Kim et al., 2006). An important finding
in our study is the association of translationally repressed, polyanosome-free FMRP–mRNP complexes with microtu-
bules (Figures 3 and 4). In the absence of FMRP, these mRNAs may be actively translated in the soma rather than
repressed and transported into the dendrite. Because FMRP associates with polyribosomes in the synapse (Feng et al.,
1997b), the translationally repressed FMRP–mRNP particles must be derepressed for local translation upon synaptic
activation. The FMRP–polyribosome association may further control translation elongation/termination based on the
phosphorylation status of FMRP (Ceman et al., 2003). Hence, the presence of FMRP in translationally dormant mRNPs for
microtubule-dependent transport and in elongating polyribosomes in dendrites/synapses may represent two distinct
phases for FMRP to control local protein synthesis. Although the I304N-FMRP-mRNP's are efficiently transported into
dendrites (Figure 7), the mRNAs in these complexes may never be activated for translation at the synapse, or they may be
misregulated for translation due to the lack of functional FMRP on polyribosomes. This may explain why the I304N
mutant FMRP fails to rescue the synaptic defects when introduced into Fmr1 postsynaptic neurons (Pfeiffer and Huber,
2007).

Figure 7. Kinetic analysis of GFP-I304N-FMRP granules by using FRAP with or without disruption of microtubule dynamics. Primary
rat hippocampal neurons (9 DIV) transfected with GFP-FMRP-
I304N for 8 h were treated with DHPG before subjected to FRAP analysis in the presence or absence of nocodazole. The image colors
 correspond to fluorescence intensities of GFP signal according to standard heat map format (Antar et al., 2005). A and E show a
representative neuron expressing GFP-FMRP-I304N granules in the absence and presence of nocodazole, respectively. The white box
outlines the dendritic area subjected to FRAP analysis in subsequent frames. The time-lapse images of GFP signal in mock-treated (B–D)
and nocodazole-treated (F and G) illustrate the effect of nocodazole in the efficiency of fluorescence recovery. B and F, prebleach. C and
G, postbleach. D and H, fluorescence recovery at 5 min after bleaching. Note that the dendritic GFP signal in the nocodazole-treated
cell (E and F) seems more diffuse, less granular and has a much higher somatic signal than in nontreated cells (A and B), and the less
robust recovery of fluorescence in the nocodazole-treated cell at 5 min after bleaching (H) compared with the untreated cell (D). (I)
Graphical analysis of the average percentage of recovery of GFP signal after photobleaching from neurons treated with DHPG alone
(red squares; see A–D) or DHPG and nocodazole (blue circles; see E–H), each shown with a best-fit curve. Bar, 20 μm.
At this point, the functional requirement of FMRP in mRNA transport has not been directly demonstrated. Previous studies examining dendritic mRNA localization in Fmr1 knockout mouse neurons have given conflicting results (Steward et al., 1998; Miyashiro et al., 2003), although neither study examined the stimulus-induced localization of FMRP ligands. Considering the fact that FMRP associates with microRNAs and the microRNA machinery (Jin et al., 2004), whether microRNA pathways may contribute to microtubule-dependent transport of FMRP–mRNPs is an intriguing possibility to be addressed by future studies. Delinеating the role of FMRP in these scenarios will provide important insight regarding the molecular mechanisms for the pathogenesis of fragile X mental retardation, and for the fundamental rules that govern local protein synthesis required for synaptic plasticity.

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