

# Sunrise at the Synapse: The FMRP mRNP Shaping the Synaptic Interface

## Minireview

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Recent studies provide new insight into the mechanistic function of Fragile X Mental Retardation Protein (FMRP), paving the way to understanding the biological basis of Fragile X Syndrome. While it has been known for several years that there are spine defects associated with the absence of the mRNA binding protein FMRP, it has been unclear how its absence may lead to specific synaptic defects that underlie the learning and cognitive impairments in Fragile X. One hypothesis under study is that FMRP may play a key role in the regulation of dendritically localized mRNAs, at subsynaptic sites where regulation of local protein synthesis may influence synaptic structure and plasticity. This review highlights recent progress to identify the specific mRNA targets of FMRP and assess defects in mRNA regulation that occur in cells lacking FMRP. In addition, exciting new studies on *Fmr1* knockout mice and mutant flies have begun to elucidate a key role for FMRP in synaptic growth, structure, and long-term plasticity.

Fragile X Syndrome is caused by the loss of FMRP, an mRNA binding protein with multiple binding domains, as well as nuclear localization and export sequences (reviewed in O'Donnell and Warren, 2002). Immunocytochemical studies indicate that FMRP is distributed throughout the neuron: in the nucleus, cell body, dendrites, and postsynaptic sites (O'Donnell and Warren, 2002). A working model is that FMRP binds specific mRNAs in the nucleus, forming a ribonucleoprotein complex, which is localized to the cytoplasm, transported to dendrites, and locally translated in response to appropriate stimuli (Figure 1).

It has been known for over 20 years that polyribosomes localize within dendrites and are often concentrated beneath synapses and at dendritic spines (reviewed in Steward and Schuman, 2001). This observation led to a hypothesis of synapse-regulated gene expression in which local synthesis provides a mechanism for targeting proteins to clusters of activated synapses, allowing for spatial and temporal control of synaptic structure and function. Does FMRP play a role here? As a first step to test this hypothesis, the specific mRNAs that bind FMRP must be identified.

### Defining FMRP Target mRNAs

In 2001, three *Cell* papers defined many mRNAs that bind FMRP, which encode proteins important for neuronal development and synaptic function (reviewed in Kaytor and Orr, 2001). Warren and colleagues used an immunoprecipitation and microarray approach to iden-

tify a few hundred mRNAs that associated with FMRP *in vivo* from rat brain extracts (Brown et al., 2001). Further analysis of many of these mRNAs in polyribosome fractions demonstrated their altered translational profiles using a human lymphoblastoid Fragile X cell line. The Darnell laboratory and colleagues showed that the RGG box of FMRP specifically binds to a planar mRNA structure, called a G-quartet, and that this interaction was required for mRNP complex formation *in vitro* (Darnell et al., 2001). In collaborative experiments, the investigators showed that several FMRP brain-associated mRNAs, with altered levels in polyribosomes from Fragile X cells, also have G-quartets. Of particular interest was the mRNA encoding MAP1B (Figure 2C), as a subsequent paper showed that the dFMR, the *Drosophila* homolog of FMRP, may act as a translational repressor of *futsch*, the homolog of MAP1B (Zhang et al., 2001). Since many FMRP target mRNAs identified encode proteins important for neuronal development and synaptic function, it is possible that the interactions between FMRP and these sequences occur in processes, even at synapses.

### Defining FMRP Target mRNAs In Situ

An inherent shortcoming of prior microarray approaches is the requirement to extract mRNA from cells, tissues, or fractions thereof. To understand FMRP's function in neurons, it becomes critical to test whether any of these FMRP-mRNA interactions occur in dendrites. This has now been accomplished, in another technical innovation from the Eberwine laboratory. In the preceding issue of *Neuron*, investigators from the Eberwine and Greenough laboratories describe the development and application of a novel technology for the identification of mRNA targets of the FMRP *in situ* (Miyashiro et al., 2003). The technique is called "APRA," for Antibody Positioned RNA Amplification, which involves coupling an oligonucleotide primer to a monoclonal antibody that binds to FMRP in fixed cells, positioning the primer for *in situ* transcription of the mRNAs that are presumably in the FMRP ribonucleoprotein complex. Following extraction of cDNA from cells, a second strand cDNA synthesis is performed, followed by aRNA amplification. Labeled RNA probes generated in this manner are hybridized to cDNA macroarrays, and positive cDNAs are then evaluated by filter binding and UV crosslinking. Approximately 60% of the APRA-defined mRNAs were shown to directly associate with FMRP. Of note, many of the FMRP-mRNA targets identified were not revealed by the previous study using a co-IP method (Brown et al., 2001) and vice versa. The differences between the FMRP-mRNA targets identified between these studies is likely because there is little overlap in the genes represented in the Affymetrix microarray used in the previous study with the cDNA macroarrays used here. However, when one examines only those genes that overlap between these arrays, there were several mRNA targets that were positively identified in both studies. Another important difference between the studies is the use here of neuronally enriched mRNA from cultured neurons, as opposed to using whole brain for the co-IP analysis or

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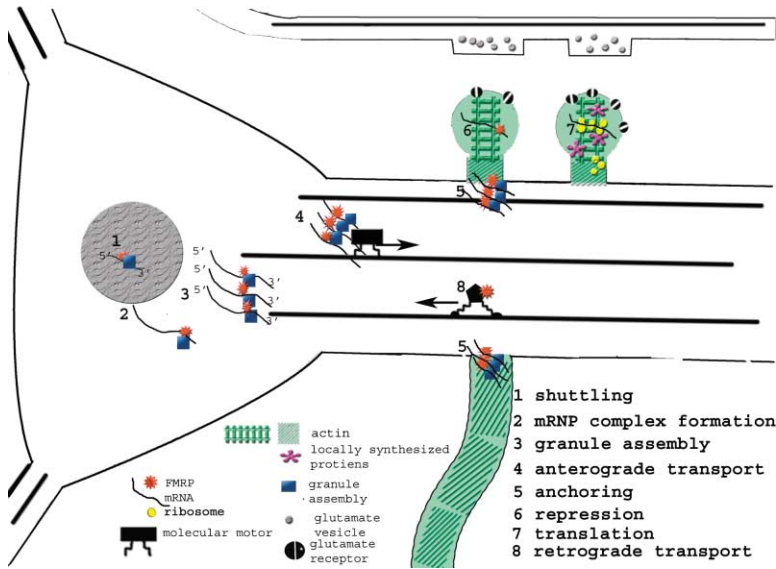


Figure 1. FMRP Here, There, and Everywhere

FMRP may bind specific mRNAs in the nucleus (1) and escort them into the cytoplasm via its NES, where it may form an mRNP complex with other proteins and mRNAs (2). The “granule” is the mRNP cargo (3), which attaches to an anterograde motor (4) and is shuttled along the dendrite to target and anchor to (5) dendritic spines or dendritic filopodia. These are sites where FMRP may play a role in synaptogenesis (in filopodial processes) or act as a repressor of protein synthesis (at the spine) (6), where it may regulate synthesis of proteins for spine structure and maintenance in an activity-dependent manner (7). Finally, FMRP may be tagged or phosphorylated in response to activity and return to the nucleus via its NLS by attaching to a retrograde motor (8) (see also Otero et al., 2002).

lymphoblastoid cells for translational defects (Brown et al., 2001).

In Miyashiro et al., several methods were used to analyze the expression and subcellular localization of FMRP-associated mRNAs and their encoded proteins. The absence of FMRP resulted in three distinct mRNA expression patterns that include reduced specific mRNA levels in hippocampal and cerebellar neurons *in vivo*, reduced mRNA levels and loss of dendritic localization, and no change in mRNA levels or localization. At the protein level, differences in the relative abundance of several FMRP targets were observed in total brain lysate and synaptosomal fractions, suggesting impairment in dendritic or synaptic targeting.

In summary, this study describes an innovative approach that has defined many FMRP target mRNAs and suggests a rather diverse role for FMRP in mRNA regulation. A major question to be addressed is whether any of these mRNAs show altered translational regulation at synapses in *Fmr1* knockout mice.

#### A Molecular Model for FMRP Repression of Synaptic mRNAs

Evidence for a function of FMRP in translational repression at the synapse has been obtained in a recent *Cell* paper from the laboratory of Bagni et al. (Zalfa et al., 2003). FMRP was shown to be in a complex with several mRNAs, including MAP1B, a known FMRP target, and CaMKII $\alpha$  and *Arc*, two dendritically localized mRNAs (Steward and Schuman, 2001). The observation that CaMKII $\alpha$  and *Arc* mRNA were detected in FMRP antibody precipitates is surprising, as these mRNAs were not detected by either of the microarray studies discussed above. In brain fractions of *Fmr1* knockout mice, the levels of these FMRP-associated mRNAs were increased in polyribosome fractions. Increased protein levels were also observed in whole-brain and/or synaptosomal fractions, providing further support for a role of FMRP in translational repression at synapses.

An interesting twist in this study is that FMRP was not shown to interact directly with these mRNAs, as discussed above, but rather indirectly via BC1 RNA (Fig-

ure 2D), a short noncoding transcript that is abundant in brain and also transported into dendrites (Muslimov et al., 1997). Recent work on BC1 from the Tiedge laboratory has revealed its role in translational repression by inhibiting formation of the 48S preinitiation complex. In addition, BC1 RNA was associated with poly (A) binding protein and eIF4A, both of which were enriched synaptically (Wang et al., 2002). In the study by Zalfa et al., they show that BC1 RNA was associated with FMRP and hypothesize that BC1 RNA forms intermolecular base pairing with specific mRNAs, suggesting that it acts as a bridge between FMRP and specific mRNAs (i.e., MAP1B). Data in support of this model were observations that an antisense oligonucleotide to the BC1 sequence, suggested to base pair with MAP1B mRNA, could inhibit the association of MAP1B mRNA with FMRP.

This study provides important new observations that show that the translation of specific mRNAs is upregulated in the absence of FMRP, possibly leading to excessive local protein synthesis at the synapse and consistent with a function for FMRP in translational repression (Laggerbauer et al., 2001). However, it was unclear why FMRP was not detected in polyribosome fractions on sucrose gradients, as has been observed by others (Feng et al., 1997).

To provide further support for the model proposed (Zalfa et al., 2003), it will be important to characterize more precisely the mechanism of BC1-facilitated FMRP repression by validation of the base-pairing theory and molecular analysis of how a BC1/FMRP complex may regulate, for example, translation initiation.

#### Fragile X Spines: An Abnormal Phenotype Providing Insight into Synaptic Function

How can altered synaptic mRNA translation cause defects in synaptic morphology observed in Fragile X Syndrome? Spine “dysmorphogenesis” was described by Purpura (1974), who showed that dendritic spines of MR patients were longer, thinner, and fewer in number than those of asymptomatic individuals. Though long and thin, spines of Fragile X patients are hyperabundant

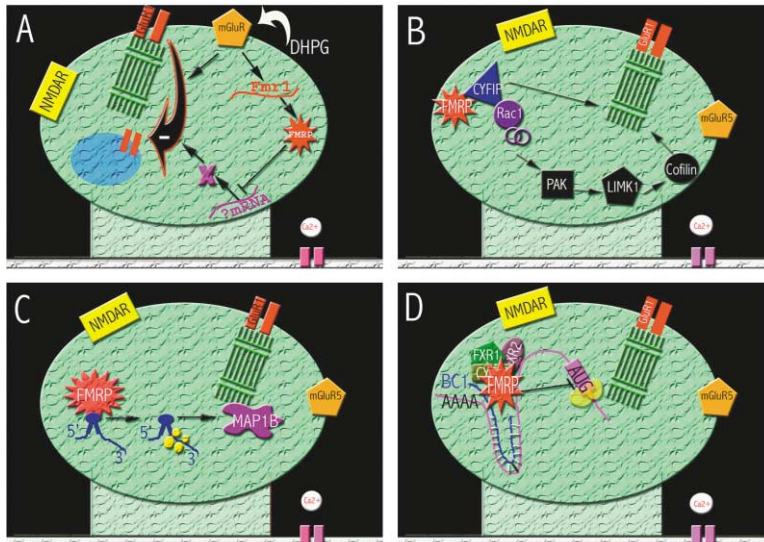


Figure 2. FMRP, A Renaissance Protein: Models for Synaptic Change

(A) Application of group 1 mGluR agonist DHPG activates the mGluR, which enhances both ionotropic receptor internalization and translation of *Fmr1* mRNA into FMRP. FMRP may act as a negative regulator or repressor of mGluR-dependent protein synthesis, which promotes receptor internalization involved in LTD (Greenough et al., 2001; Huber et al., 2002). (B) FMRP binds to CYFIP, which interacts directly with F-actin. CYFIP also binds the GTPase Rac1, which is known to regulate actin structure via its association with cofilin. Both of these interactions may lead to changes in spine structure (Bardoni and Mandel, 2002). (C) FMRP binds to the 5'UTR of MAP1B mRNA via its G-quartet structure. This may repress translation of MAP1B mRNA, which could influence local MAP1B expression and possibly microtubule and actin structure (Kaytor and Orr, 2001). (D) FMRP and BC1 mRNA may interact with target mRNAs to repress translation. Loss of FMRP can lead to aberrant translation of key synaptic proteins, which may contribute to the Fragile X phenotype (adapted from Zalfa et al., 2003).

(reviewed in Greenough et al., 2001; Fiala et al., 2002). Many investigators are examining whether morphology or density of dendritic spines underlies mechanisms of learning and memory impaired in Fragile X.

Immature spine morphology has been reported in slice cultures of the barrel cortex of *Fmr1* knockout mice during a window of 7–14 days (Nimchinsky et al., 2001). This window coincides with the critical period of plasticity for that structure, a time of intense permanent functional alteration in response to stimuli. In contrast, in dissociated 3-week-old hippocampal neurons of *Fmr1*-deficient mice, abnormally short and low-density synaptic spines were observed (Braun and Segal, 2000.) Diminished functional contacts in the hippocampus accompanied these changes, as did diminished excitatory synaptic currents. The transient phenotype observed in both of these studies, gone shortly after the structures exit their respective critical periods, may point toward a crucial role for FMRP in mediating plastic events. Is this signature spine morphology of Fragile X due to a compensatory mechanism of the cell (Fiala et al., 2002) or a direct result of FMRP loss? Assuming a direct role for FMRP, two processes, synaptogenesis and spine maintenance, are implicated in FX syndrome, a one-protein disease, suggesting a dual role for this single protein.

#### **An Activity-Dependent Role for FMRP in Dendritic Spines**

Since FMRP has been shown to localize to spines via immunoelectron microscopy (Feng et al., 1997), an activity-dependent role for FMRP expression and localization has been sought. Several groups utilized cortical synaptosomal preparations to demonstrate that FMRP levels increase in response to both barrel cortex stimulation in vivo and mGluR activation in vitro. Additionally, polyribosomes fractionated on sucrose gradients demonstrate a shift after they are probed for *Fmr1* mRNA

post mGluR stimulation (reviewed, references cited in Greenough et al., 2001). The latter study suggests that *Fmr1* mRNA is present and translated at the synapse and provides an additional means for FMRP localization and concentration at the synapse in an activity-dependent manner. Outside of the cortex, immunohistochemical studies in the hippocampus, a structure important in learning and memory, have shown that FMRP levels can increase after rearing mice in complex environments (for review, see Greenough et al., 2001).

#### **FMRP in Synaptic Plasticity: Where Spine Structure May Directly Regulate Function**

Because of the activity-dependence of FMRP, and the MR that results without the protein, many groups examine different models of long-term plasticity. An exciting recent study has reported enhanced mGluR-dependent long-term depression (LTD) in hippocampal slices from *Fmr1* knockout mice (Huber et al., 2002; see also Figure 2A). These investigators previously showed that group 1 mGluR5 signaling induced a protein synthesis-dependent form of LTD that is dependent on postsynaptic protein synthesis and involves internalization of AMPA and NMDA receptors (Snyder et al., 2001). Bear and Huber suggest a link between enhanced mGluR-dependent LTD and spine elongation; one possibility is that activation of mGluR receptors can lead to a local increase of calcium from internal stores, which has been associated with lengthening of dendritic spines (Vanderklish and Edelman, 2002). They suggest that FMRP may negatively regulate or repress mRNAs involved in mGluR-dependent LTD. In the absence of FMRP, excessive LTD ensues, which may be a prelude to synapse elimination and associated spine defects. One model is that excessive synapse elimination, caused by increased LTD, leads to an increase in filopodia, which is a response to the loss of spines.

Though impaired LTP has not been reported in the

hippocampus, Li et al. (2002) have used tetanic stimulation to induce LTP in the cortex, cerebellum, and hippocampus of *Fmr1*-deficient mice at 8–10 weeks. They found in the cortex that there was both reduced cortical LTP and a reduced number of GluR1 receptors. While no microarray studies have indicated direct changes in GluR1 mRNA levels in *Fmr1*-deficient animals, it is possible that GluR1 regulation occurs through other proteins whose mRNAs are differentially regulated in the knockout.

#### **FMRP Maintenance of the Cytoskeletal Architecture**

Other roles for FMRP regulation of spine morphology are suggested by its binding interactions with proteins and mRNAs that shape cytoskeletal architecture. First, FMRP binds to Cytoplasmic FMRP Interacting Protein 1 (CYFIP1) (for review, see Bardoni and Mandel, 2002), which, in turn, binds to Rac-1, a member of the subgroup of the Ras superfamily of GTPases. These GTPases are “switches” that regulate the actin cytoskeleton and are implicated in dendritic modeling and remodeling (Figure 2B). When Rac-1 is constitutively active, it is associated with an abnormal increase in spine density, a known phenotypic abnormality in Fragile X Syndrome. Rac-1 is thought to be involved in the maturation and maintenance of dendritic spines (Nakayama et al., 2000).

Another candidate role for FMRP changing synaptic structure comes from recent work at the *Drosophila* NMJ. Futsch rearranges cytoskeletal loops that form during division of the synaptic bouton. The light chain of MAP1B, the mammalian homolog to Futsch, binds actin stress fibers in vivo (Togel et al., 1998). In the *dfmr1* mutant, there is an increase in growth and branching and an overall increase in bouton number, by up to 50%, that seems to result from increased Futsch expression (Gao, 2002). Evoked synaptic transmission was also increased in the *dfmr1* mutant NMJ. In contrast, overexpression of dFMR1 in both pre- and postsynaptic compartments leads to a decrease in bouton number. It was concluded that synaptic structure and function is regulated by dFMR1 repression of Futsch mRNA (Gao, 2002). Once again, FMRP is linked to the structural architecture of the synapse and its function.

#### **A Role for FMRP in the Axon**

Observations of axonal defects in *dfmr1* null mutants suggest a possible local function for FMRP in the regulation of axonal outgrowth and/or presynaptic structure. Indeed, it was shown by immunofluorescence (Zhang et al., 2001) that dFMR1 is localized to axons in normal flies. It is possible that the abnormal phenotypes are a direct consequence of the absence of regulated Futsch mRNA translation by dFMR1 in axons. Observations of mRNA localization and translation in axons have been given new attention; this is no longer a phenomenon restricted to invertebrates (Guiditta et al., 2002). It would be exciting to identify a specific function for FMRP in axons.

#### **Conclusions**

FMRP, a renaissance protein, appears to have multifarious functions: repressor, shuttle, adaptor, and transporter. FMRP is no longer part of an elusive mRNP, since many interesting specific mRNA targets and associated mRNAs have been identified. The challenge is to characterize the molecular mechanism(s) of how FMRP may

regulate local mRNA translation in response to synaptic signaling pathways involved in long-term plasticity. One obvious course is to find whether the specific defects in spine morphology and/or synaptic plasticity discussed in this review can be shown to be dependent on altered translation of FMRP-associated mRNAs, of which there are many interesting candidates to now study.

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