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Fragile X Mental Retardation Protein: A Paradigm for Translational Control by RNA-Binding Proteins

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Abstract

Translational control is a common mechanism used to regulate gene expression and occur in bacteria to mammals. Typically in translational control, an RNA-binding protein binds to a unique sequence in the mRNA to regulate protein synthesis by the ribosomes. Alternatively, a protein may bind to or modify a translation factor to globally regulate protein synthesis by the cell. Here, we review translational control by the fragile X mental retardation protein (FMRP), the absence of which causes the neurological disease, fragile X syndrome (FXS).

Keywords

fragile X syndrome; fragile X mental retardation protein; ribosome; translation; RNA; neuron

Introduction

There are more than 400 RNA-binding proteins (RBP) encoded in the human genome and they regulate diverse processes such as mRNA transcription, splicing, polyadenylation, transport, translation, and RNA degradation (Cook et al., 2011). One of the important functions of RBPs is the control of protein synthesis. Translational control by RBPs is essential during early embryonic development (Lasko, 2012), cell cycle progression (Roux and Topisirovic, 2012; Ruggero, 2013), in learning and memory (Costa-Mattioli et al., 2009; Darnell and Richter, 2012), and in the life cycle of some viruses (Walsh et al., 2013). Defects in translational control can lead to diseases such as autism (Gkogkas et al., 2013; Santini et al., 2013), cancer (Hsieh et al., 2012), and intellectual disability (Bhakar et al., 2012). Fragile X syndrome (FXS) is a classic example of translational control gone awry. FXS is the most frequent form of inherited intellectual disability and is caused by the altered expression of a single gene located on the X chromosome, the Fragile X Mental Retardation 1 (*FMR1*) gene (Fu et al., 1991; Pieretti et al., 1991; Verkerk et al., 1991). The 5' – untranslated region of the *FMR1* gene normally contains about 50 CGG trinucleotide

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repeats, which is expanded to over 200 repeats in patients with FXS (Figure 1A). The expanded CGG repeats is hypermethylated leading to the transcriptional silencing of the *FMR1* gene (Sutcliffe et al., 1992). A recent study showed that *FMR1* mRNA transcript with the expanded CGG repeats hybridizes to the complementary CGG-repeat portion of the *FMR1* gene to silence the gene (Colak et al., 2014).

The *FMR1* gene encodes the fragile X mental retardation protein (FMRP), which is an RNA-binding protein that is highly expressed in the brain and reproductive organs (Ashley et al., 1993; Hinds et al., 1993; Siomi et al., 1993). The absence of FMRP in the neurons leads to subtle developmental abnormalities in the brain such as neurons having immature, thin and highly branched dendritic spines (Irwin et al., 2001). These changes to the spine morphology is thought to be due to lack of translational control by FMRP causing excessive protein synthesis in the spines and is proposed to be the molecular basis for FXS. FXS patients exhibit difficulties in learning, social shyness, hyperactivity, increased susceptibility to seizures, hypersensitivity to sensory stimuli, macroorchidism, motor incoordination, sleep disturbances, and autistic behavior (Garber et al., 2008). In fact, FXS is the leading monogenic cause for autism spectrum disorder. Interestingly, 55 to 200 CGG repeats cause the increased transcription of *FMR1* gene and has been linked to fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X-related primary ovarian insufficiency (FXPOI) (Figure 1A) (Greco et al., 2006; Sherman, 2000). However, in FXTAS and FXPOI, the FMRP level is lower than normal and the disease is caused by the excess *FMR1* transcripts forming aggregates (Jin et al., 2003; Kenneson et al., 2001). Thus, *FMR1* gene expression has to be precisely regulated to prevent disease demonstrating a critical role for this gene in the normal development of the brain and reproductive organs.

FMR1 gene is highly conserved across species making it possible to use several animal models to study FXS. For example, knockout (KO) mouse lacking FMRP have been created and these mice show many of the same phenotypes observed in FXS patients, including disrupted learning and increased susceptibility to seizures (Bakker et al., 1994). Additionally, the neurons of *Fmr1* KO mice exhibit immature, thin and branched spine morphology (Grossman et al., 2006). Similarly, *Drosophila* with the fly ortholog, *dFmr1* inactivated display defects in learning and memory, and reduced courtship interest (Dockendorff et al., 2002; Morales et al., 2002; Zhang et al., 2001). The neurons of these *dFmr1* mutant fruit flies also show synaptic morphology similar to that observed in FXS patients and KO mice (Pan et al., 2004). These animal models are invaluable for understanding the neuronal defects in FXS and for dissecting the function of FMRP.

FMRP Related Proteins: FXR1 and FXR2

Humans and mice have two autosomal paralogs of FMRP designated as fragile X related 1 and 2 (FXR1 and FXR2) proteins (Zhang et al., 1995). *Fxr1* and *Fxr2* genes are absent in *Drosophila*. FXR1 and FXR2 have 60% overall amino acid identity to FMRP and are also highly expressed in the brain (Siomi et al., 1996; Tamanini et al., 1997). All three proteins form homodimers and also associate with one another to form heterodimers (Siomi et al., 1996). However, the functional implications for homodimers versus heterodimers are unknown. The function of FXR1 appears to be distinct from FMRP. FXR1 is essential for

skeletal and cardiac muscle development and inactivation of *Fxr1* gene in mice results in death shortly after birth (Mientjes et al., 2004). In humans, the altered expression of *FXR1* has been linked to facioscapulohumeral muscular dystrophy (FSHD), the most prevalent form of muscular dystrophy (Davidovic et al., 2008). The function of *FXR2* appears to be somewhat similar to *FMRP*. Inactivation of *FXR2* is not lethal and mice lacking *FXR2* show some behavioral phenotypes that are similar to those observed in mice lacking the *Fmr 1* gene (Bontekoe et al., 2002; Spencer et al., 2006; Zhang et al., 2009). However, *FXR2* cannot completely compensate for the loss of *FMRP* (Zhang et al., 2009). Thus, all three proteins are essential for the normal development of mammals.

Structure of FMRP

FMRP has three RNA binding motifs: two hnRNP K-homology (KH1 and KH2) motifs and one arginine-glycine-glycine (RGG) rich motif (Figure 1B) (Ashley et al., 1993; Siomi et al., 1994; Siomi et al., 1993). Crystal structure of the tandem KH1–KH2 domains showed that both KH domains are composed of three anti-parallel β -strands next to three α -helices with most of the hydrophobic residues located between the β -sheet and the α -helices (Figure 2A) (Valverde et al., 2007). Interestingly, a patient with a severe case of FXS had normal CGG repeat length but had a mutation of one of these hydrophobic residues (Ile304Asn in human *FMRP*) (De Boule et al., 1993). The Ile304Asn mutation is located in the KH2 domain and decreases overall protein stability indicating that the hydrophobic core is essential for the correct folding and function of *FMRP* (Valverde et al., 2008). More recently, screening FXS patients by sequencing the *FMR1* gene led to the identification of a missense mutation (Gly266Glu) in the KH1 domain of *FMRP* (Myrick et al., 2014).

The solution structure of an RGG peptide derived from *FMRP* bound to the SC1 GQ forming aptamer was recently solved revealing how the RGG domain of *FMRP* may interact with GQ sequences in mRNAs (Figure 2B) (Phan et al., 2011). The aptamer folds into an unusual GQ structure having a duplex stem. The GQ is formed by three stacked G-tetrads and a mixed tetrad formed by A, G and U at the duplex-quadruplex junction. The RGG peptide binds to the duplex-quadruplex junction and interacts mainly with the major groove of the duplex segment. Interestingly, the RGG peptide adopts a sharp turn at the duplex-quadruplex junction and the fold is stabilized by two arginines (Arg10 and Arg15) of the RGG peptide forming hydrogen bonds with the major groove edges of two guanines in the duplex segment. This interaction by the arginines of the RGG peptide is important for the sequence specific recognition of the SC1 GQ aptamer.

FMRP also has a nuclear localization signal (NLS), nuclear export signal (NES), and two tandem Agenet domains (Figure 1B) (Adams-Cioaba et al., 2010; Adinolfi et al., 2003; Eberhart et al., 1996; Ramos et al., 2006). The solution structure of the tandem Agenet domains showed that each domain folds into a bent four-stranded antiparallel β -sheet, with a fifth strand going across the cavity of the β -sheet thereby giving the individual domains a globular shape (Figure 2C) (Ramos et al., 2006). The Agenet domains generally bind to trimethylated lysine residues of histones and a recent study suggests that *FMRP* may play a role in chromatin remodeling (Alpatov et al., 2014). Since the structure of the full-length

protein has not been solved, it is unknown how the different domains of FMRP are organized with respect to each other in three-dimension.

Role of FMRP in mRNA Transport and in Translational Control

The presence of the NLS and NES permits FMRP to shuttle between the nucleus and the cytoplasm (Feng et al., 1997b). The ability to shuttle between the nucleus and the cytoplasm may allow FMRP to transport mRNAs out of the nucleus. Studies suggest that FMRP interacts with the bulk mRNA exporter Tap/NXF1 protein to facilitate the movement of mRNAs from the nucleus to the cytoplasm (Kim et al., 2009). During transport the mRNA cargoes are not translated possibly because they are coated by RNA binding proteins making them inaccessible to the translational machinery. Once the mRNAs reach their correct destinations in the cell, the proteins responsible for mRNA export dissociates and are recycled. However, it is unclear whether FMRP is also released or remain bound to the mRNAs to repress their translation.

Since FXS is caused by deregulated translation of specific mRNAs in the neurons, a major research effort over the last two decades has been to identify mRNA targets that are regulated by FMRP. Dreyfuss and co-workers showed that FMRP binds strongly to poly(G) RNA and weakly to poly(U) RNA but does not bind to poly(A) RNA and poly (C) RNA (Siomi et al., 1993; Wan et al., 2000). *In vitro* selection experiments identified a G-quadruplex (GQ) forming aptamer sequence that binds with high affinity to the RGG domain and another aptamer that forms a pseudoknot structure that binds to the KH2 domain of FMRP (Darnell et al., 2005; Darnell et al., 2001). These results suggest that FMRP binds to mRNAs that possess GQ or pseudoknot forming sequences and spurred the search for authentic mRNA target sequences that are recognized by FMRP. Early studies estimated that nearly 4% of brain mRNAs bind to FMRP (Ashley et al., 1993). Some of the identified target mRNAs have putative GQ sequences and code for proteins that are critical for synaptic modifications (Brown et al., 2001), which is consistent with the idea that the loss of translational regulation of these mRNAs by FMRP will lead to changes in the structure and function of the synapse.

Darnell and co-workers used high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) to identify neuronal mRNA regulated by FMRP in the mouse brain (Darnell et al., 2011). Interestingly, the ≈ 800 unique mRNAs that they identified do not contain GQ or pseudoknot forming sequences, although it is very difficult to rule this out completely using current sequence analysis tools. Recently, Tuschal and co-workers used photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) to identify ≈ 6000 mRNAs that could potentially bind to FMRP (Ascano et al., 2012). Their data suggested that the KH1 domain binds to WGGA sequence and the KH2 domain binds to ACUK sequence (in which W = A or U and K = G or U) in the target mRNAs. However, our studies showed that the WGGA and ACUK sequences are not essential for translational inhibition by FMRP (Chen et al., 2014). Finally, a high-throughput *in vitro* method to determine the RNA binding specificity of RBPs indicated that the KH domains of human and *Drosophila* FMRP bind to KGACARG and AHGGACR (in which R = A or G and H = A, U or C), respectively (Ray et al., 2013).

Independent analysis of the above four FMRP datasets indicated that the GACR core sequence is highly enriched, whereas the ACUK sequence is not enriched in the mRNA targets that are common to the four datasets (Suhl et al., 2014). The WGGGA sequence is weakly enriched but is clustered possibly because they form GQ structures and bind to the RGG domain of FMRP (Suhl et al., 2014). Thus, the RNA sequence or structure recognized by the KH domains of FMRP is not fully clear.

Validated mRNA Targets of FMRP

As described above, new high-throughput methods have identified several thousand mRNAs that potentially bind to FMRP. However, only a small number of these mRNA targets have been biochemically validated to interact with FMRP. They include *Fmr1*, *Map1B*, *Sema3F*, and *Psd95* mRNAs (Santoro et al., 2012). Microtubule associated protein 1b (*Map1B*) mRNA is probably one of the best-characterized targets of FMRP. MAP1B protein is an important component of the microtubule-based synaptic cytoskeleton and is required for dendritic development and synaptic growth (Zhang et al., 2001). Genetic and biochemical analysis showed that FMRP is a translational repressor of *futsch*, the *Drosophila* homolog of the mammalian *Map1B* (Zhang et al., 2001). *Map1B* mRNA has a putative GQ-forming sequence in its 5'-UTR and biophysical experiments showed that the RGG domain of FMRP binds to the isolated GQ sequence with high affinity ($K_D \approx 20$ nM) (Menon et al., 2008). Several of the other validated mRNA targets of FMRP also possess putative GQ sequences (Santoro et al., 2012). Taken together, these results indicate that mRNAs having GQ sequences are authentic targets of FMRP. Nevertheless, it is possible that FMRP also interacts indirectly with some mRNAs that do not have GQ sequence by either binding to adapter proteins or noncoding RNAs that recognize these mRNAs.

Mechanism of Translational Repression by FMRP

Eukaryotic mRNAs have a 7-methyl guanosine cap structure at the 5' end (m^7G) that is required for efficient translation initiation by the canonical pathway. The m^7G cap binds to eukaryotic translation initiation factor 4E (eIF4E) to form the eIF4F cap-binding complex (composed of eIF4E, eIF4G, and eIF4A) that ultimately recruits the ribosome to begin protein synthesis (Aitken and Lorsch, 2012). The interaction of eIF4E with eIF4G is key to forming the eIF4F complex at the 5' end of capped mRNAs and this step is often regulated by eIF4E-binding proteins (4E-BPs). Interestingly, studies showed that Cytoplasmic FMRP Interacting Protein (CYFIP1) interacts with both FMRP and eIF4E to repress translation initiation (Napoli et al., 2008). The proposed model is that FMRP binds to select mRNAs and recruits CYFIP1, which then binds to eIF4E at the 5'- m^7G cap and blocks interaction with eIF4G resulting in the inhibition of translation initiation (Figure 3A).

A second proposed mechanism for translational control by FMRP makes use of the microRNA pathway. The microRNA pathway is an important mechanism for the post-transcriptional control of gene expression. microRNAs are conserved ≈ 22 nucleotides long RNAs that bind to complementary sequences present in the 3'-UTR of mRNAs to inhibit translation and/or promote the degradation of the mRNAs. FMRP was shown to associate with essential components of the microRNA pathway such as Dicer and Argonaute 2

proteins as well as with specific microRNAs (Caudy et al., 2002; Ishizuka et al., 2002; Plante et al., 2006). Furthermore, FMRP was shown to promote the formation of an Argonaute 2-microRNA-125a inhibitory complex on postsynaptic density protein 95 (PSD-95) mRNA (Edbauer et al., 2010; Muddashetty et al., 2011). Formation of this inhibitory complex on PSD-95 mRNA depends on phosphorylation of FMRP. Interestingly, mGluR signaling leads to a decrease in the level of FMRP phosphorylation followed by the release of the microRNA inhibitory complex on PSD-95 mRNA and activation of translation (Muddashetty et al., 2011). Taken together, these results suggest that FMRP could recruit specific microRNA complexes to inhibit the translation of specific mRNAs in an activity-dependent manner (Figure 3B).

A third mechanism for translational control is by FMRP directly interacting with the translating ribosome (Figure 3B). Numerous studies have shown that FMRP associates with polyribosomes in the cell (Corbin et al., 1997; Feng et al., 1997a; Ishizuka et al., 2002; Khandjian et al., 1996; Mazroui et al., 2003; Siomi et al., 1996; Tamanini et al., 1996). Additionally, elegant studies by Darnell and co-workers showed that FMRP stalls translation during the elongation phase by forming large structures composed of ribosomes, mRNA, FMRP and other proteins (Darnell et al., 2011). However, it was unclear whether this association of FMRP with polyribosomes is direct or mediated by the mRNA or other proteins as part of a translationally repressed large mRNA-protein complex (mRNP complex). Interestingly, FMRP co-immunoprecipitated with the large ribosomal subunit proteins L5 and L18 (ribosomal proteins are labeled as per the new nomenclature, see (Jenner et al., 2012)) along with 5S rRNA from whole cell extracts suggesting that FMRP could bind directly to the ribosome (Ishizuka et al., 2002). We developed a quantitative method to analyze the interaction of FMRP with purified ribosomes in the absence of mRNA and other proteins. Our studies showed that FMRP directly binds with high affinity to the ribosome ($K_D \approx 20$ nM) and also crosslinked to ribosomal protein L5 indicating a specific interaction with the ribosome (Chen et al., 2014). Furthermore, cryoEM studies showed that the KH domains of FMRP bind in the space between the large and small ribosomal subunits, close to ribosomal protein L5 (Figure 4) (Chen et al., 2014). Interestingly, the C-terminal RGG domain of FMRP is more solvent exposed and is in the vicinity of the mRNA A-site codon. The cryoEM data suggest that the binding of FMRP to the ribosome would stall protein synthesis by blocking the binding of tRNAs and elongation factors to the ribosome (Figure 3B) (Chen et al., 2014). However, a structure of an authentic translationally repressed ribosome•FMRP complex is needed to understand how both FMRP and the P site tRNA are accommodated in the ribosome and whether the RGG domain interacts with a specific sequence motif in the mRNA. It is also not clear how FMRP is released from the ribosome to relieve the translational inhibition. One possibility is that in the activated synapse, FMRP is polyubiquitinated and rapidly degraded by the proteasome to relieve the translational inhibition (Hou et al., 2006; Nalavadi et al., 2012). Another possibility is that post-translational modification of FMRP (phosphorylation or methylation) will modulate its affinity for the ribosome (Blackwell and Ceman, 2011; Ceman et al., 2003; Denman, 2002; Dolzhanskaya et al., 2006; Siomi et al., 2002). More studies are needed to understand how the interaction of FMRP with the ribosome is modulated by sequences or structures in the mRNA and by modifications to FMRP.

Although three models for translational control by FMRP are discussed above, it is not necessary that FMRP use exclusively only one mechanism. In fact, it is more likely that FMRP regulates translation by multiple mechanisms depending on the mRNA, its location within the cell, and the functional step that is being regulated. For example, FMRP may block translation initiation by recruiting CYFIP 1 while the mRNA is being transported from the nucleus to distal sites such as the dendrites of a neuron. Once the mRNA reaches the correct location in the cell, FMRP bound to the mRNA may interact with the ribosome to stall translation, forming large mRNP complexes. Synaptic activity may lead to post-translational modification of FMRP and relieve the translational inhibition by FMRP. FMRP may then use the microRNA pathway to fine-tune the translation of specific mRNAs in an activity-dependent manner.

Could FMRP Function also as a General Repressor of Translation?

The direct binding of FMRP to the ribosome suggests that FMRP could repress translation in an mRNA independent manner (Chen et al., 2014). FMRP at high concentrations may bind directly to the ribosomes to bring the overall increased translational activity of the stimulated synapse back to basal level so that synaptic homeostasis is achieved and the synapse is ready for the next stimulation (Turrigiano, 2008). Indeed, the levels of FMRP in activated synapses rapidly increase for a short time and then decrease to steady state levels (Hou et al., 2006; Weiler et al., 1997). It will be interesting to determine whether FMRP may also function as a general repressor of protein synthesis to bring the synapse back to the resting state.

The mGluR Theory and Drugs for the Treatment of FXS

Long-term depression (LTD) is an activity-dependent decrease of synaptic strength that lasts hours or longer after stimulation of the neuron. Several neurotransmitters and neurotransmitter receptors are involved in LTD depending on the type of neuron. Metabotropic glutamate receptor (mGluR) LTD is a major form of LTD, which depends on local protein synthesis at the synapse and leads to the internalization of α -amino-3-hydroxyl-4-isoxazole propionic acid (AMPA) receptors (Carroll et al., 1999; Huber et al., 2000; Kemp and Bashir, 1999). Importantly, the activation of mGluR overcomes FMRP-dependent inhibition of local protein synthesis, which is essential for LTD. According to the mGluR theory, in the absence of FMRP, mGluR-signaling pathway is excessively stimulated leading to uncontrolled local protein synthesis at the synapse and is the root cause for the FXS phenotype (Bear et al., 2004). The mGluR theory predicts that decreasing mGluR activation may be beneficial to FXS patients. Consistent with the mGluR hypothesis, studies indicate that LTD is exaggerated in *Fmr1* KO mice and does not require new protein synthesis (Huber et al., 2002; Nosyreva and Huber, 2006). Agonists of mGluR also trigger local protein synthesis at the synapse in normal mice but not in *Fmr1* KO mice (Muddashetty et al., 2007). Taken together, these data suggest that mGluR-LTD is impaired in FXS because mRNAs that are normally repressed by FMRP are inappropriately expressed even without mGluR activation. These promising results have spurred the search for drugs that antagonize mGluR activation. Notably, the mGluR antagonist 2-methyl-6-

(phenylethynyl)-pyridine (MPEP) can partly rescue the FXS phenotype in *Drosophila* and mouse models of FXS (McBride et al., 2005; Yan et al., 2005).

Many drugs trials are underway to treat FXS that target the mGluR signaling pathway and gamma-aminobutyric acid (GABA) receptor signaling (which is downregulated in FXS). Fenobam, a mGluR5 antagonist was shown to be effective in *Fmr1* KO mice and is being tested in humans with FXS (Berry-Kravis et al., 2009). Agonists of the GABA signaling pathway such as acamprosate and ganaxolone are also being tested for treatment of FXS (Lozano et al., 2014). Additionally, a drug trial is underway to use minocycline, an analogue of the tetracycline antibiotic, to treat children with FXS (Leigh et al., 2013). Minocycline is an inhibitor of matrix metalloproteinase-9 (MMP-9), a secreted extracellular endopeptidase that can alter spine morphology (Dziembowska et al., 2013). Absence of FMRP increases the expression of MMP-9 in neurons and the increased MMP-9 activity is proposed to be responsible for the defective spine morphology observed in FXS. Indeed, inhibiting MMP-9 activity promotes normal spine morphology in *Fmr1* KO mice (Bilousova et al., 2009). Surprisingly, lovastatin, a drug widely used for the treatment of high cholesterol, appears to reduce some of the FXS phenotype in *Fmr1* KO mice (Osterweil et al., 2013). Lovastatin is proposed to normalize the increases in protein synthesis in *Fmr1* KO mice by suppressing the activity of the Ras-dependent MAP kinases, ERK1/2. Thus, a number of new drugs that target steps upstream of protein synthesis are in clinical trials for the treatment of FXS. Since FMRP could also bind directly to the ribosome to regulate protein synthesis, identifying novel compounds that interact with the ribosome to directly modulate protein synthesis may be a new avenue to treat patients with FXS.

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References

- Adams-Cioaba MA, Guo Y, Bian C, Amaya MF, Lam R, Wasney GA, Vedadi M, Xu C, Min J. Structural studies of the tandem Tudor domains of fragile X mental retardation related proteins FXR1 and FXR2. *PLoS One*. 2010; 5:e13559. [PubMed: 21072162]
- Adinolfi S, Ramos A, Martin SR, Dal Piaz F, Pucci P, Bardoni B, Mandel JL, Pastore A. The N-terminus of the fragile X mental retardation protein contains a novel domain involved in dimerization and RNA binding. *Biochemistry*. 2003; 42:10437–10444. [PubMed: 12950170]
- Aitken CE, Lorsch JR. A mechanistic overview of translation initiation in eukaryotes. *Nat Struct Mol Biol*. 2012; 19:568–576. [PubMed: 22664984]
- Alpatov R, Lesch BJ, Nakamoto-Kinoshita M, Blanco A, Chen S, Stutzer A, Armache KJ, Simon MD, Xu C, Ali M, et al. A Chromatin-Dependent Role of the Fragile X Mental Retardation Protein FMRP in the DNA Damage Response. *Cell*. 2014; 157:869–881. [PubMed: 24813610]
- Ascano M Jr, Mukherjee N, Bandaru P, Miller JB, Nusbaum JD, Corcoran DL, Langlois C, Munschauer M, Dewell S, Hafner M, et al. FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature*. 2012; 492:382–386. [PubMed: 23235829]
- Ashley CT Jr, Wilkinson KD, Reines D, Warren ST. FMR1 protein: conserved RNP family domains and selective RNA binding. *Science*. 1993; 262:563–566. [PubMed: 7692601]

- Bakker C, Verheij C, Willemsen R, van der Helm R, Oerlemans F. Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. *Cell*. 1994; 78:23–33. [PubMed: 8033209]
- Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004; 27:370–377. [PubMed: 15219735]
- Berry-Kravis E, Hessel D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV, et al. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet*. 2009; 46:266–271. [PubMed: 19126569]
- Bhakar AL, Dolen G, Bear MF. The pathophysiology of fragile X (and what it teaches us about synapses). *Annu Rev Neurosci*. 2012; 35:417–443. [PubMed: 22483044]
- Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, Ethell IM. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J Med Genet*. 2009; 46:94–102. [PubMed: 18835858]
- Blackwell E, Ceman S. A new regulatory function of the region proximal to the RGG box in the fragile X mental retardation protein. *J Cell Sci*. 2011; 124:3060–3065. [PubMed: 21868366]
- Bontekoe CJ, McIlwain KL, Nieuwenhuizen IM, Yuva-Paylor LA, Nellis A, Willemsen R, Fang Z, Kirkpatrick L, Bakker CE, McAninch R, et al. Knockout mouse model for Fxr2: a model for mental retardation. *Hum Mol Genet*. 2002; 11:487–498. [PubMed: 11875043]
- Brown V, Jin P, Ceman S, Darnell JC, O'Donnell WT, Tenenbaum SA, Jin X, Feng Y, Wilkinson KD, Keene JD, et al. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell*. 2001; 107:477–487. [PubMed: 11719188]
- Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC. Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nat Neurosci*. 1999; 2:454–460. [PubMed: 10321250]
- Caudy AA, Myers M, Hannon GJ, Hammond SM. Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev*. 2002; 16:2491–2496. [PubMed: 12368260]
- Ceman S, O'Donnell WT, Reed M, Patton S, Pohl J, Warren ST. Phosphorylation influences the translation state of FMRP-associated polyribosomes. *Hum Mol Genet*. 2003; 12:3295–3305. [PubMed: 14570712]
- Chen E, Sharma MR, Shi X, Agrawal RK, Joseph S. Fragile x mental retardation protein regulates translation by binding directly to the ribosome. *Mol Cell*. 2014; 54:407–417. [PubMed: 24746697]
- Colak D, Zaninovic N, Cohen MS, Rosenwaks Z, Yang WY, Gerhardt J, Disney MD, Jaffrey SR. Promoter-bound trinucleotide repeat mRNA drives epigenetic silencing in fragile X syndrome. *Science*. 2014; 343:1002–1005. [PubMed: 24578575]
- Cook KB, Kazan H, Zuberi K, Morris Q, Hughes TR. RBPDB: a database of RNA-binding specificities. *Nucleic Acids Res*. 2011; 39:D301–D308. [PubMed: 21036867]
- Corbin F, Bouillon M, Fortin A, Morin S, Rousseau F, Khandjian EW. The fragile X mental retardation protein is associated with poly(A)+ mRNA in actively translating polyribosomes. *Hum Mol Genet*. 1997; 6:1465–1472. [PubMed: 9285783]
- Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. Translational control of long-lasting synaptic plasticity and memory. *Neuron*. 2009; 61:10–26. [PubMed: 19146809]
- Darnell JC, Fraser CE, Mostovetsky O, Stefani G, Jones TA, Eddy SR, Darnell RB. Kissing complex RNAs mediate interaction between the Fragile-X mental retardation protein KH2 domain and brain polyribosomes. *Genes Dev*. 2005; 19:903–918. [PubMed: 15805463]
- Darnell JC, Jensen KB, Jin P, Brown V, Warren ST, Darnell RB. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell*. 2001; 107:489–499. [PubMed: 11719189]
- Darnell JC, Richter JD. Cytoplasmic RNA-binding proteins and the control of complex brain function. *Cold Spring Harbor perspectives in biology*. 2012; 4:a012344. [PubMed: 22723494]
- Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraser CE, Stone EF, Chen C, Fak JJ, Chi SW, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell*. 2011; 146:247–261. [PubMed: 21784246]
- Davidovic L, Sacconi S, Bechara EG, Delplace S, Allegra M, Desnuelle C, Bardoni B. Alteration of expression of muscle specific isoforms of the fragile X related protein 1 (FXR1P) in

facioscapulohumeral muscular dystrophy patients. *J Med Genet.* 2008; 45:679–685. [PubMed: 18628314]

De Boulle K, Verkerk AJ, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van den Bos F, de Graaff E, Oostra BA, Willems PJ. A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat Genet.* 1993; 3:31–35. [PubMed: 8490650]

Denman RB. Methylation of the arginine-glycine-rich region in the fragile X mental retardation protein FMRP differentially affects RNA binding. *Cell Mol Biol Lett.* 2002; 7:877–883. [PubMed: 12378270]

Dockendorff TC, Su HS, McBride SM, Yang Z, Choi CH, Siwicki KK, Sehgal A, Jongens TA. *Drosophila* lacking dfmr1 activity show defects in circadian output and fail to maintain courtship interest. *Neuron.* 2002; 34:973–984. [PubMed: 12086644]

Dolzanskaya N, Merz G, Aletta JM, Denman RB. Methylation regulates the intracellular protein-protein and protein-RNA interactions of FMRP. *J Cell Sci.* 2006; 119:1933–1946. [PubMed: 16636078]

Dziembowska M, Pretto DI, Janusz A, Kaczmarek L, Leigh MJ, Gabriel N, Durbin-Johnson B, Hagerman RJ, Tassone F. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. *Am J Med Genet A.* 2013; 161A:1897–1903. [PubMed: 23824974]

Eberhart DE, Malter HE, Feng Y, Warren ST. The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. *Hum Mol Genet.* 1996; 5:1083–1091. [PubMed: 8842725]

Edbauer D, Neilson JR, Foster KA, Wang CF, Seeburg DP, Batterton MN, Tada T, Dolan BM, Sharp PA, Sheng M. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. *Neuron.* 2010; 65:373–384. [PubMed: 20159450]

Feng Y, Absher D, Eberhart DE, Brown V, Malter HE, Warren ST. FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. *Mol Cell.* 1997a; 1:109–118. [PubMed: 9659908]

Feng Y, Gutekunst CA, Eberhart DE, Yi H, Warren ST, Hersch SM. Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. *J Neurosci.* 1997b; 17:1539–1547. [PubMed: 9030614]

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG Jr, Warren ST, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell.* 1991; 67:1047–1058. [PubMed: 1760838]

Garber KB, Visootsak J, Warren ST. Fragile X syndrome. *Eur J Hum Genet.* 2008; 16:666–672. [PubMed: 18398441]

Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, Weatherill DB, Vasuta C, Yee S, Truitt M, Dallaire P, et al. Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature.* 2013; 493:371–377. [PubMed: 23172145]

Greco CM, Berman RF, Martin RM, Tassone F, Schwartz PH, Chang A, Trapp BD, Iwahashi C, Brunberg J, Grigsby J, et al. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). *Brain : a journal of neurology.* 2006; 129:243–255. [PubMed: 16332642]

Grossman AW, Aldridge GM, Weiler IJ, Greenough WT. Local protein synthesis and spine morphogenesis: Fragile X syndrome and beyond. *J Neurosci.* 2006; 26:7151–7155. [PubMed: 16822971]

Hinds HL, Ashley CT, Sutcliffe JS, Nelson DL, Warren ST, Housman DE, Schalling M. Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. *Nat Genet.* 1993; 3:36–43. [PubMed: 8490651]

Hou L, Antion MD, Hu D, Spencer CM, Paylor R, Klann E. Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. *Neuron.* 2006; 51:441–454. [PubMed: 16908410]

Hsieh AC, Liu Y, Edlind MP, Ingolia NT, Janes MR, Sher A, Shi EY, Stumpf CR, Christensen C, Bonham MJ, et al. The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature.* 2012; 485:55–61. [PubMed: 22367541]

- Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A*. 2002; 99:7746–7750. [PubMed: 12032354]
- Huber KM, Kayser MS, Bear MF. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science*. 2000; 288:1254–1257. [PubMed: 10818003]
- Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy F, Willems PJ, Cras P, Kozlowski PB, et al. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am J Med Genet*. 2001; 98:161–167. [PubMed: 11223852]
- Ishizuka A, Siomi MC, Siomi H. A *Drosophila* fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev*. 2002; 16:2497–2508. [PubMed: 12368261]
- Jenner L, Melnikov S, de Loubresse NG, Ben-Shem A, Iskakova M, Urzhumtsev A, Meskauskas A, Dinman J, Yusupova G, Yusupov M. Crystal structure of the 80S yeast ribosome. *Current opinion in structural biology*. 2012; 22:759–767. [PubMed: 22884264]
- Jin P, Zarnescu DC, Zhang F, Pearson CE, Lucchesi JC, Moses K, Warren ST. RNA-mediated neurodegeneration caused by the fragile X premutation rCGG repeats in *Drosophila*. *Neuron*. 2003; 39:739–747. [PubMed: 12948442]
- Kemp N, Bashir ZI. Induction of LTD in the adult hippocampus by the synaptic activation of AMPA/kainate and metabotropic glutamate receptors. *Neuropharmacology*. 1999; 38:495–504. [PubMed: 10221753]
- Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet*. 2001; 10:1449–1454. [PubMed: 11448936]
- Khandjian EW, Corbin F, Woerly S, Rousseau F. The fragile X mental retardation protein is associated with ribosomes. *Nat Genet*. 1996; 12:91–93. [PubMed: 8528261]
- Kim M, Bellini M, Ceman S. Fragile X mental retardation protein FMRP binds mRNAs in the nucleus. *Mol Cell Biol*. 2009; 29:214–228. [PubMed: 18936162]
- Lasko P. mRNA localization and translational control in *Drosophila* oogenesis. *Cold Spring Harbor perspectives in biology*. 2012; 4
- Leigh MJ, Nguyen DV, Mu Y, Winarni TI, Schneider A, Chechi T, Polussa J, Doucet P, Tassone F, Rivera SM, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile x syndrome. *Journal of developmental and behavioral pediatrics : JDBP*. 2013; 34:147–155. [PubMed: 23572165]
- Lozano R, Hare EB, Hagerman RJ. Modulation of the GABAergic pathway for the treatment of fragile X syndrome. *Neuropsychiatric disease and treatment*. 2014; 10:1769–1779. [PubMed: 25258535]
- Mazroui R, Huot ME, Tremblay S, Boilard N, Labelle Y, Khandjian EW. Fragile X Mental Retardation protein determinants required for its association with polyribosomal mRNPs. *Hum Mol Genet*. 2003; 12:3087–3096. [PubMed: 14532325]
- McBride SM, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D, Sehgal A, Siwicki KK, Dockendorff TC, Nguyen HT, et al. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a *Drosophila* model of fragile X syndrome. *Neuron*. 2005; 45:753–764. [PubMed: 15748850]
- Menon L, Mader SA, Mihailescu MR. Fragile X mental retardation protein interactions with the microtubule associated protein 1B RNA. *RNA*. 2008; 14:1644–1655. [PubMed: 18579868]
- Mientjes EJ, Willemsen R, Kirkpatrick LL, Nieuwenhuizen IM, Hoogeveen-Westerveld M, Verweij M, Reis S, Bardoni B, Hoogeveen AT, Oostra BA, et al. Fxr1 knockout mice show a striated muscle phenotype: implications for Fxr1p function in vivo. *Hum Mol Genet*. 2004; 13:1291–1302. [PubMed: 15128702]
- Morales J, Hiesinger PR, Schroeder AJ, Kume K, Verstreken P, Jackson FR, Nelson DL, Hassan BA. *Drosophila* fragile X protein, DFXR, regulates neuronal morphology and function in the brain. *Neuron*. 2002; 34:961–972. [PubMed: 12086643]
- Muddashetty RS, Kelic S, Gross C, Xu M, Bassell GJ. Dysregulated metabotropic glutamate receptor-dependent translation of AMPA receptor and postsynaptic density-95 mRNAs at synapses in a mouse model of fragile X syndrome. *J Neurosci*. 2007; 27:5338–5348. [PubMed: 17507556]

- Muddashetty RS, Nalavadi VC, Gross C, Yao X, Xing L, Laur O, Warren ST, Bassell GJ. Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Mol Cell*. 2011; 42:673–688. [PubMed: 21658607]
- Myrick LK, Nakamoto-Kinoshita M, Lindor NM, Kirmani S, Cheng X, Warren ST. Fragile X syndrome due to a missense mutation. *Eur J Hum Genet*. 2014
- Nalavadi VC, Muddashetty RS, Gross C, Bassell GJ. Dephosphorylation-induced ubiquitination and degradation of FMRP in dendrites: a role in immediate early mGluR-stimulated translation. *J Neurosci*. 2012; 32:2582–2587. [PubMed: 22357842]
- Napoli I, Mercaldo V, Boyl PP, Eleuteri B, Zalfa F, De Rubeis S, Di Marino D, Mohr E, Massimi M, Falconi M, et al. The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell*. 2008; 134:1042–1054. [PubMed: 18805096]
- Nosyreva ED, Huber KM. Metabotropic receptor-dependent long-term depression persists in the absence of protein synthesis in the mouse model of fragile X syndrome. *J Neurophysiol*. 2006; 95:3291–3295. [PubMed: 16452252]
- Osterweil EK, Chuang SC, Chubykin AA, Sidorov M, Bianchi R, Wong RK, Bear MF. Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of fragile X syndrome. *Neuron*. 2013; 77:243–250. [PubMed: 23352161]
- Pan L, Zhang YQ, Woodruff E, Broadie K. The Drosophila fragile X gene negatively regulates neuronal elaboration and synaptic differentiation. *Curr Biol*. 2004; 14:1863–1870. [PubMed: 15498496]
- Phan AT, Kuryavyi V, Darnell JC, Serganov A, Majumdar A, Ilin S, Raslin T, Polonskaia A, Chen C, Clain D, et al. Structure-function studies of FMRP RGG peptide recognition of an RNA duplex-quadruplex junction. *Nat Struct Mol Biol*. 2011; 18:796–804. [PubMed: 21642970]
- Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell*. 1991; 66:817–822. [PubMed: 1878973]
- Plante I, Davidovic L, Ouellet DL, Gobeil LA, Tremblay S, Khandjian EW, Provost P. Dicer-derived microRNAs are utilized by the fragile X mental retardation protein for assembly on target RNAs. *J Biomed Biotechnol*. 2006; 2006:64347. [PubMed: 17057366]
- Ramos A, Hollingworth D, Adinolfi S, Castets M, Kelly G, Frenkiel TA, Bardoni B, Pastore A. The structure of the N-terminal domain of the fragile X mental retardation protein: a platform for protein-protein interaction. *Structure*. 2006; 14:21–31. [PubMed: 16407062]
- Ray D, Kazan H, Cook KB, Weirauch MT, Najafabadi HS, Li X, Gueroussov S, Albu M, Zheng H, Yang A, et al. A compendium of RNA-binding motifs for decoding gene regulation. *Nature*. 2013; 499:172–177. [PubMed: 23846655]
- Roux PP, Topisirovic I. Regulation of mRNA translation by signaling pathways. *Cold Spring Harbor perspectives in biology*. 2012; 4
- Ruggero D. Translational control in cancer etiology. *Cold Spring Harbor perspectives in biology*. 2013; 5
- Santini E, Huynh TN, MacAskill AF, Carter AG, Pierre P, Ruggero D, Kaphzan H, Klann E. Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature*. 2013; 493:411–415. [PubMed: 23263185]
- Santoro MR, Bray SM, Warren ST. Molecular mechanisms of fragile X syndrome: a twenty-year perspective. *Annu Rev Pathol*. 2012; 7:219–245. [PubMed: 22017584]
- Sherman SL. Premature ovarian failure among fragile X premutation carriers: parent-of-origin effect? *Am J Hum Genet*. 2000; 67:11–13. [PubMed: 10848491]
- Siomi H, Choi M, Siomi MC, Nussbaum RL, Dreyfuss G. Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell*. 1994; 77:33–39. [PubMed: 8156595]
- Siomi H, Siomi MC, Nussbaum RL, Dreyfuss G. The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. *Cell*. 1993; 74:291–298. [PubMed: 7688265]
- Siomi MC, Higashijima K, Ishizuka A, Siomi H. Casein kinase II phosphorylates the fragile X mental retardation protein and modulates its biological properties. *Mol Cell Biol*. 2002; 22:8438–8447. [PubMed: 12446764]

- Siomi MC, Zhang Y, Siomi H, Dreyfuss G. Specific sequences in the fragile X syndrome protein FMR1 and the FXR proteins mediate their binding to 60S ribosomal subunits and the interactions among them. *Mol Cell Biol*. 1996; 16:3825–3832. [PubMed: 8668200]
- Spencer CM, Serysheva E, Yuva-Paylor LA, Oostra BA, Nelson DL, Paylor R. Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. *Hum Mol Genet*. 2006; 15:1984–1994. [PubMed: 16675531]
- Suhl JA, Chopra P, Anderson BR, Bassell GJ, Warren ST. Analysis of FMRP mRNA target datasets reveals highly associated mRNAs mediated by G-quadruplex structures formed via clustered WGGA sequences. *Hum Mol Genet*. 2014; 23:5479–5491. [PubMed: 24876161]
- Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet*. 1992; 1:397–400. [PubMed: 1301913]
- Tamanini F, Meijer N, Verheij C, Willems PJ, Galjaard H, Oostra BA, Hoogeveen AT. FMRP is associated to the ribosomes via RNA. *Hum Mol Genet*. 1996; 5:809–813. [PubMed: 8776596]
- Tamanini F, Willemsen R, van Unen L, Bontekoe C, Galjaard H, Oostra BA, Hoogeveen AT. Differential expression of FMR1, FXR1 and FXR2 proteins in human brain and testis. *Hum Mol Genet*. 1997; 6:1315–1322. [PubMed: 9259278]
- Turrigiano GG. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell*. 2008; 135:422–435. [PubMed: 18984155]
- Valverde R, Edwards L, Regan L. Structure and function of KH domains. *FEBS J*. 2008; 275:2712–2726. [PubMed: 18422648]
- Valverde R, Pozdnyakova I, Kajander T, Venkatraman J, Regan L. Fragile X mental retardation syndrome: structure of the KH1–KH2 domains of fragile X mental retardation protein. *Structure*. 2007; 15:1090–1098. [PubMed: 17850748]
- Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*. 1991; 65:905–914. [PubMed: 1710175]
- Walsh D, Mathews MB, Mohr I. Tinkering with translation: protein synthesis in virus-infected cells. *Cold Spring Harbor perspectives in biology*. 2013; 5:a012351. [PubMed: 23209131]
- Wan L, Dockendorff TC, Jongens TA, Dreyfuss G. Characterization of dFMR1, a Drosophila melanogaster homolog of the fragile X mental retardation protein. *Mol Cell Biol*. 2000; 20:8536–8547. [PubMed: 11046149]
- Weiler IJ, Irwin SA, Klintsova AY, Spencer CM, Brazelton AD, Miyashiro K, Comery TA, Patel B, Eberwine J, Greenough WT. Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Proc Natl Acad Sci U S A*. 1997; 94:5395–5400. [PubMed: 9144248]
- Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology*. 2005; 49:1053–1066. [PubMed: 16054174]
- Zhang J, Hou L, Klann E, Nelson DL. Altered hippocampal synaptic plasticity in the FMR1 gene family knockout mouse models. *J Neurophysiol*. 2009; 101:2572–2580. [PubMed: 19244359]
- Zhang Y, O'Connor JP, Siomi MC, Srinivasan S, Dutra A, Nussbaum RL, Dreyfuss G. The fragile X mental retardation syndrome protein interacts with novel homologs FXR1 and FXR2. *EMBO J*. 1995; 14:5358–5366. [PubMed: 7489725]
- Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, Rubin GM, Broadie K. Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. *Cell*. 2001; 107:591–603. [PubMed: 11733059]

Highlights

- Fragile X Syndrome (FXS) is the most common form of inherited mental retardation
- FXS is caused by the absence of the Fragile X Mental Retardation Protein (FMRP)
- FMRP is an RNA-binding protein that regulates protein synthesis
- FMRP binds to G-quadruplex structures in mRNAs
- FMRP inhibits protein synthesis by binding directly to the ribosome

A



Normal: <55 CGG repeats

Premutation (FXTAS & FXPOI): 55 - 200 CGG repeats

Full mutation (FXS): >200 CGG repeats

B

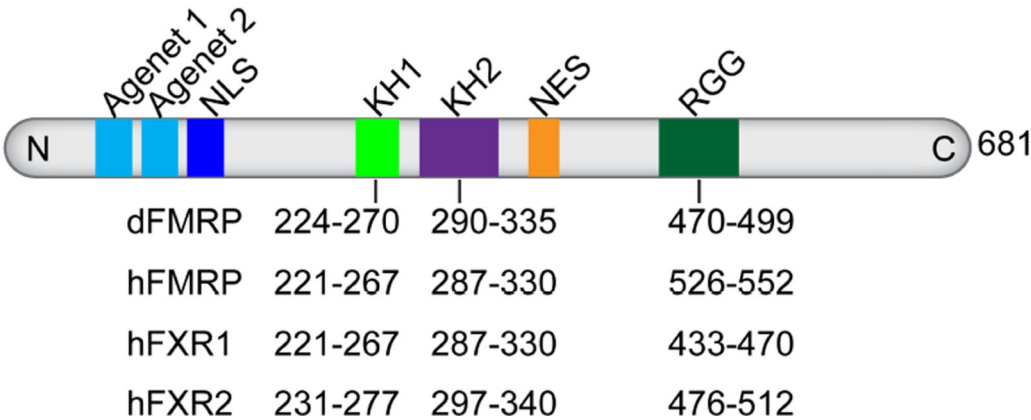


Figure 1. *FMR1* gene and domain organization of FMRP

(A) *FMR1* gene showing CGG repeat expansion. Normal individuals have less than 55 CGG trinucleotide repeats in the 5'-untranslated region (UTR) of the *FMR1* gene. Individuals with premutation have 55 to 200 CGG repeats, which has been linked to fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X-related primary ovarian insufficiency (FXPOI). FXS patients have greater than 200 CGG repeats. ATG indicates the start codon for the *FMR1* gene. (B) Domain organization of FMRP. FMRP has two Agetet motifs (cyan), two KH motifs (KH1 in light green and KH2 in purple), a RGG box (olive green), nuclear localization sequence (NLS, blue) and nuclear export sequence (NES, orange). The amino acid residues corresponding to the KH1, KH2 and RGG motifs are indicated below

the figure for *Drosophila* FMRP (dFMRP), human FMRP (hFMRP), human FXR1 (hFXR1) and human FXR2 (hFXR2).

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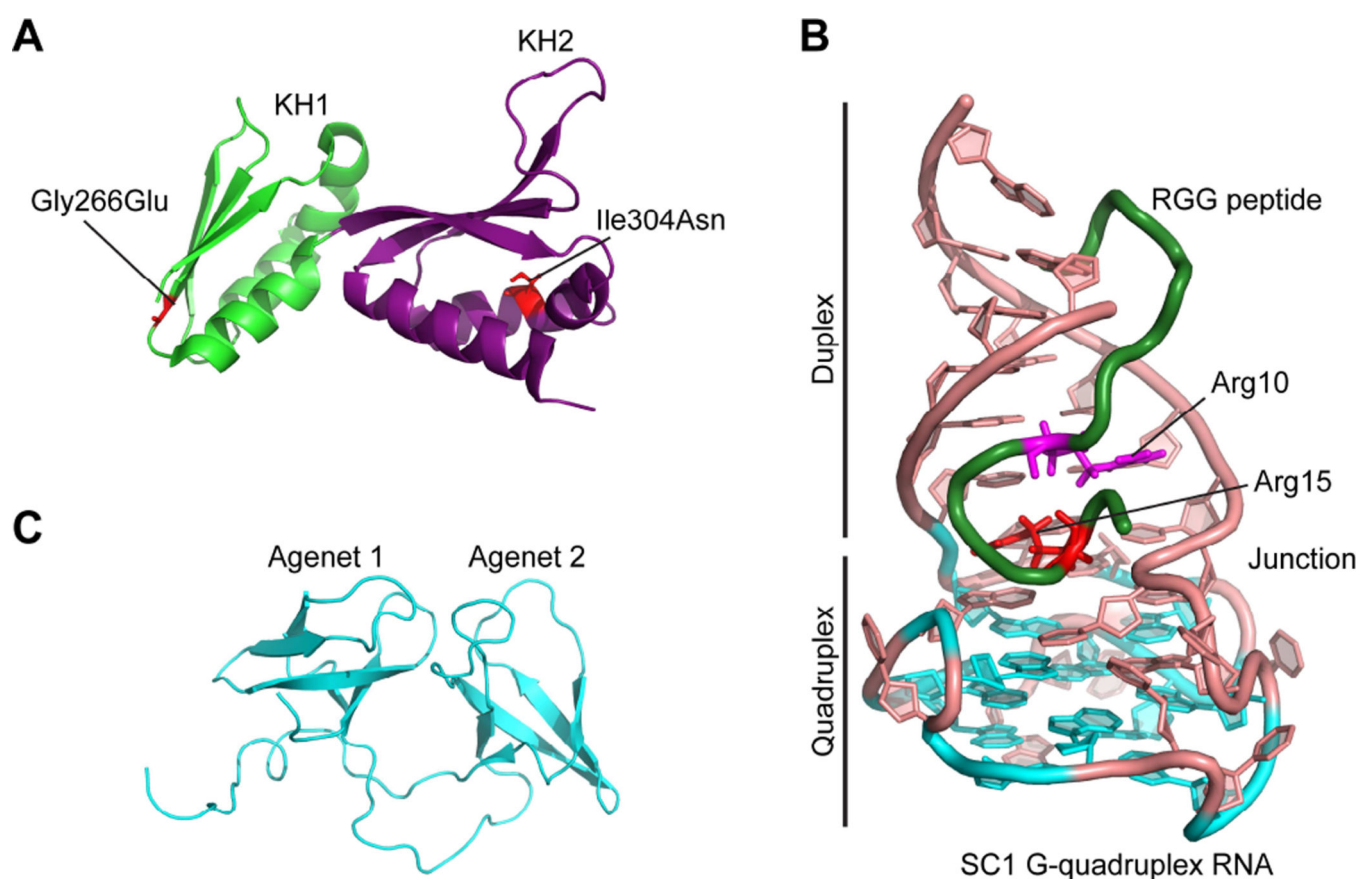


Figure 2. Structures of FMRP functional domains

(A) Structure of the tandem KH domains (PDB code: 2QND). KH1 is in green showing the Gly266Glu missense mutation (red) and KH2 is in purple showing the Ile304Asn missense mutation (red). (B) Structure of the SC1 G-quadruplex forming RNA bound to a peptide sequence derived from the RGG box of FMRP (PDB code: 2LA5). RNA is in salmon and the RGG peptide is in olive green. Guanine bases involved in forming the G-tetrads are in cyan. The two arginines of the RGG peptide that are important for recognizing the SC1 RNA are shown in magenta (Arg10) and red (Arg15). (C) Structure of the tandem Agenet motifs (PDB code: 2BKD). See text for more details.

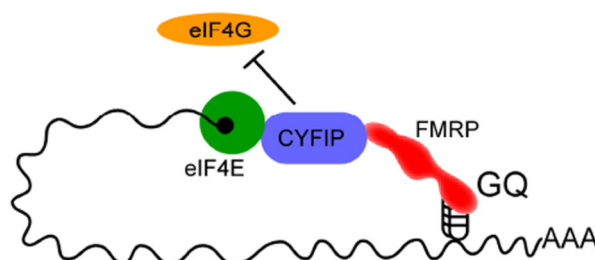
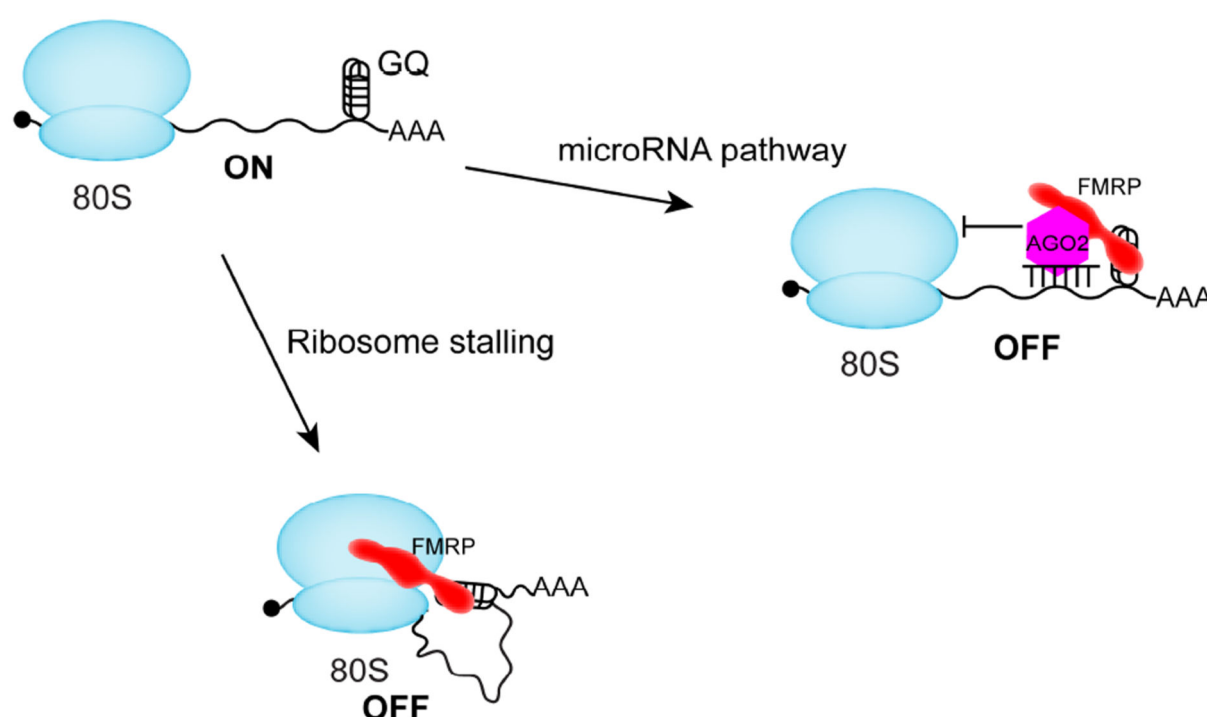
A**B**

Figure 3. Models for translational control by FMRP

(A) FMRP (red) inhibits translation initiation by binding to a unique sequence or structure in the mRNA and recruiting CYFIP (blue). For simplicity, the mRNA motif recognized by FMRP is shown as a G-quadruplex (GQ) structure. CYFIP interacts with eIF4E (green) and blocks eIF4G (orange) from binding to eIF4E to form the eIF4F complex. (B) FMRP inhibits translation elongation by binding to a GQ structure in the mRNA and recruiting microRNAs, Argonaute 2 (AGO2), and Dicer to form the RNA-induced silencing complex. Alternatively, FMRP binds to a GQ structure in the mRNA and then binds directly to the ribosome to stall translation.

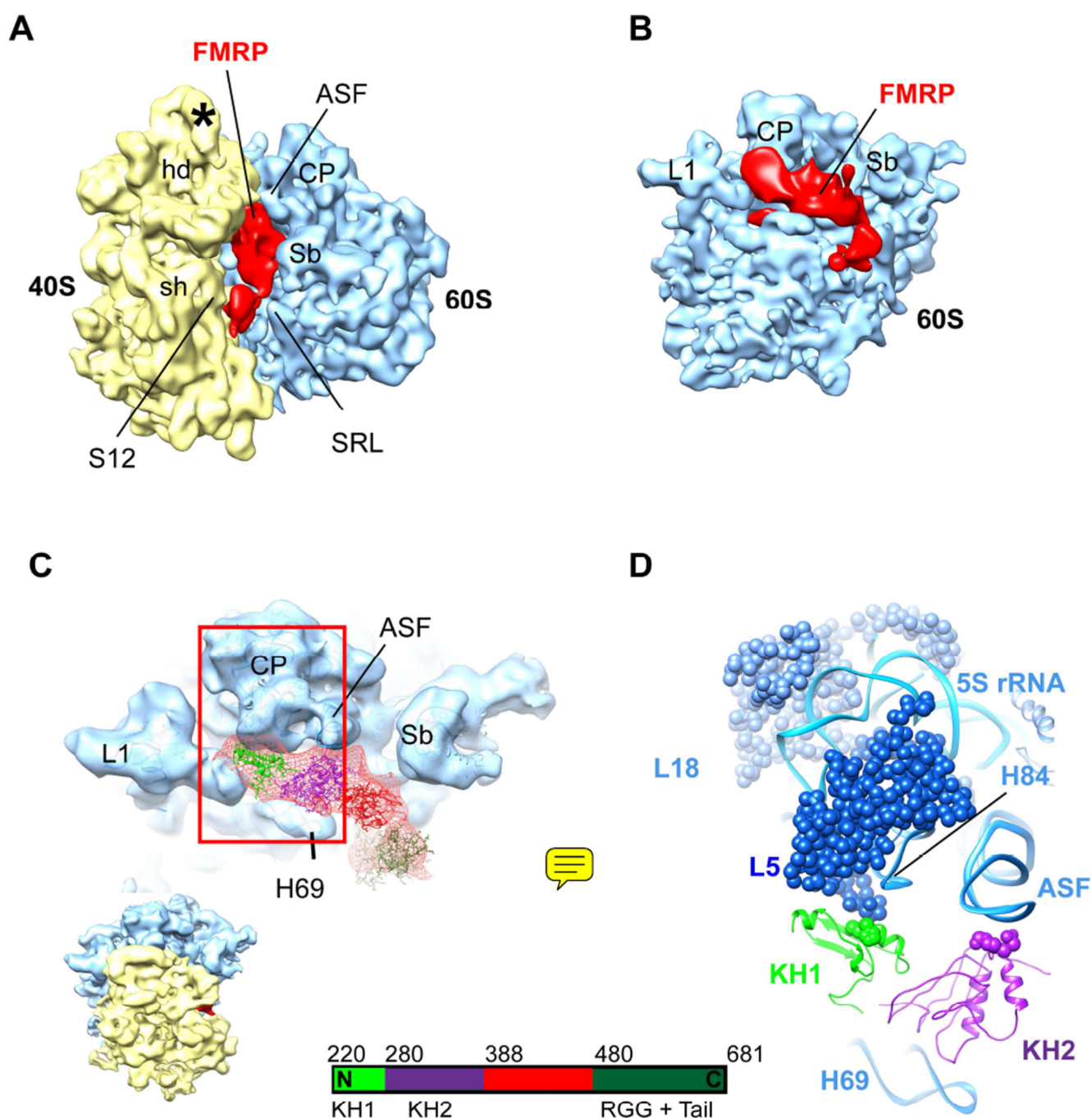


Figure 4. Cryo-EM structure of FMRP bound to the ribosome

(A) Structure of *Drosophila* FMRP bound to 80S ribosome. Cryo-EM density for the *Drosophila* FMRP (which was truncated by 25 kD from the N-terminus; red), 40S small ribosomal subunit (yellow) and 60S large ribosomal subunit (blue) are shown. (B) The structure shown in (A) after rotation by +90° around the vertical axis and without showing the 40S ribosomal subunit. (C) Overall binding position of FMRP on the 60S subunit. The inset shows the orientation of the ribosome. (D) Close-up of the interaction between FMRP and the 60S subunit. Regions approximately corresponding to KH1, KH2 and the RGG plus

C-terminal tail of FMRP are indicated in light green, purple, and olive green, respectively. Labels: hd and sh are “head” and “shoulder”, respectively of the 40S; ASF, A-site finger or 28S rRNA helix 38; CP, central protuberance; Sb, L7/L12 stalk base; SRL, α -sarcin-ricin stem-loop; L1, L1 protein protuberance; L5 and L18 are shown as space-filling models; H69 and H84, 28S rRNA helices 69 and 84, respectively.

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