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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...
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 \textit{FMR1} gene (Sutcliffe et al., 1992). A recent study showed that \textit{FMR1} mRNA transcript with the expanded CGG repeats hybridizes to the complementary CGG-repeat portion of the \textit{FMR1} gene to silence the gene (Colak et al., 2014).

The \textit{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 \textit{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 \textit{FMR1} transcripts forming aggregates (Jin et al., 2003; Kenneson et al., 2001). Thus, \textit{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.

\textit{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 \textit{Fmr1} KO mice exhibit immature, thin and branched spine morphology (Grossman et al., 2006). Similarly, \textit{Drosophila} with the fly ortholog, \textit{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 \textit{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). \textit{Fxr1} and \textit{Fxr2} genes are absent in \textit{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 homeodimers 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 \textit{Fxr1} gene in mice results in death shortly after birth (Mientjes et al., 2004). In humans, the altered expression of \textit{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 \textit{Fmr1} 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.

\textbf{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 \(\beta\)-strands next to three \(\alpha\)-helices with most of the hydrophobic residues located between the \(\beta\)-sheet and the \(\alpha\)-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 Boulle 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 \textit{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 \(\beta\)-sheet, with a fifth strand going across the cavity of the \(\beta\)-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 WGGA 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 (KD ≈ 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 (m7G) that is required for efficient translation initiation by the canonical pathway. The m7G 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'-m7G 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 ≈ 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-hydroxy-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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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
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 Agenet 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).
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.
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.