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Aberrant RNA translation in fragile X syndrome: From FMRP mechanisms to emerging therapeutic strategies

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Abstract

Research in the past decades has unfolded the multifaceted role of Fragile X mental retardation protein (FMRP) and how its absence contributes to the pathophysiology of Fragile X syndrome (FXS). Excess signaling through group 1 metabotropic glutamate receptors is commonly observed in mouse models of FXS, which in part is attributed to dysregulated translation and downstream signaling. Considering the wide spectrum of cellular and physiologic functions that loss of FMRP can affect in general, it may be advantageous to pursue disease mechanism based treatments that directly target translational components or signaling factors that regulate protein synthesis. Various FMRP targets upstream and downstream of the translational machinery are therefore being investigated to further our understanding of the molecular mechanism of RNA and protein synthesis dysregulation in FXS as well as test their potential role as therapeutic interventions to alleviate FXS associated symptoms. In this review, we will broadly discuss recent advancements made towards understanding the role of FMRP in translation regulation, new pre-clinical animal models with FMRP targets located at different levels of the translational and signal transduction pathways for therapeutic intervention as well as future use of stem cells to model FXS associated phenotypes.

Keywords

Fragile X syndrome; mRNA translation; mRNA localization; RNA binding protein; Dendritic spine; iPSC

1. Introduction

Fragile x syndrome (FXS) is the most common form of inherited intellectual disability and the leading monogenetic cause of autism. Since the discovery of the *FMR1* gene, which encodes for the fragile x mental retardation protein (FMRP), the field has progressed from bench to bedside and back to bench. There were major advances to understand the role of FMRP as an mRNA binding protein that regulates translation, among other aspects of post-transcriptional regulation. In addition, we learned that FMRP has non-canonical functions to

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regulate ion channels through protein-protein interactions. Major progress on the neurobiology of FMRP function revealed dysregulation of protein synthesis in the brain of animal models of FXS and human patient cells. One well-studied feature in FXS animal models is the dysregulated signaling through metabotropic glutamate receptors (mGlu1/5) leading to impairments in protein synthesis, synaptic structure, development and function. Based on these studies, clinical trials in FXS patients sought to dampen excess protein synthesis and exaggerated signal transduction by targeting mGlu5; however, their outcome was not successful in meeting primary endpoints. While there are many reasons for failed clinical trials, we need to further understand the basic biology of FMRP function and develop improved and more specific disease mechanism targeted therapeutic strategies. This mini-review will focus on recent advances in the biology of FMRP as an RNA binding protein and consequences to the loss of RNA regulation in fragile x syndrome. This reductionist approach has informed the development of potential pharmacologic interventions to rescue a wide range of molecular, cellular, physiologic and behavioral phenotypes in animal models. It is also clear that that the field needs to incorporate human *in vitro* models to study disease mechanism and test emerging therapies. A new chapter of translational research and neurotherapeutics will be forthcoming.

2. FMRP regulates mRNA translation

FMRP has been shown to repress the translation of specific mRNAs, many of which encode proteins that regulate synaptic structure and function, such as *PSD-95, Arc*, and *Shank1* (Ascano et al., 2012; Darnell et al., 2011; Pasciuto and Bagni, 2014). FMRP associated mRNAs have been identified through a variety of experimental methods. Early studies identified the G-quadruplex structures as a reoccurring feature of FMRP targets through both FMRP-RNA co-immunoprecipitation (RIP-CHIP) (Brown et al., 2001) and oligonucleotide-based systematic evolution of ligands by exponential enrichment (SELEX) (Darnell et al., 2001). Fourteen of the RNAs identified to bind FMRP were also altered in their polyribosome distribution in lymphoblastoid cell lines derived from fragile x patients. FMRP can regulate the translation of target mRNAs through multiple mechanisms, and in addition to ribosome stalling, can also repress translation by association with the RNA-induced silencing complex (RISC) (Muddashetty et al., 2011) and other mechanisms (Richter et al., 2015). Observations of increased basal rates of protein synthesis and loss of stimulus-induced protein synthesis in both the mouse model of FXS (Gross et al., 2010; Liu et al., 2012; Sharma et al., 2010) and cells from FXS patients (Gross and Bassell, 2012) further provide evidence for translational dysregulation as underlying core mechanism of FXS.

Mouse models of FXS have demonstrated dysregulated metabotropic glutamate receptor 5 (mGlu5) activation of protein synthesis and signal transduction (Bear et al., 2004; Gross et al., 2010; Osterweil et al., 2010; Sharma et al., 2010). This dysregulation specifically impacts mammalian target of rapamycin, mTOR signaling pathways (Gross et al., 2015a; Sharma et al., 2010). There are several downstream molecules contributing to altered mGlu5 mediated signal transduction that are dysregulated in FXS, including PI3K, ERK and S6K; some but not all are direct FMRP targets (Richter et al., 2015). mGlu5 signaling activates long-term depression (LTD), a specific form of long term synaptic plasticity that is

dependent on new protein synthesis. mGlu5 dependent LTD is enhanced in mice lacking FMRP (Huber et al., 2002), yet is protein synthesis independent in *Fmr1* KO mice (Nosyreva and Huber, 2006). Taken together these studies suggest a potential direct connection between dysregulated protein synthesis and impaired learning, memory and cognitive function in FXS. A molecular understanding of the underlying mechanisms by which FMRP can regulate mRNA translation, protein synthesis and related signaling pathways is necessary to better understand the pathology in human FXS patients.

3. FMRP directly binds RNA

It is well known that FMRP can interact directly with multiple mRNAs to regulate their translation. Therefore, many studies have focused on identifying common sequence motifs amongst confirmed FMRP target RNAs (Ascano et al., 2012; Darnell et al., 2001; Darnell et al., 2011; Suhl et al., 2014). Some of these studies demonstrate an enrichment of *cis*-element-specific binding sites in the 5′ and 3′ untranslated regions (UTRs) of FMRP targets, such as G-quadruplex structures formed by WGGA sequences (Ascano et al., 2012; Stefanovic et al., 2015; Suhl et al., 2014; Zhang et al., 2014). Direct binding of FMRP to several mRNA targets containing G-quadruplex structures has been shown using biophysical methods(Zanotti et al., 2006). Most recently, the G-quadruplex structures within the 3′UTR of *Shank1* mRNA was shown to be bound directly by FMRP (Zhang et al., 2014). FMRP mediated repression of Shank1 mRNA was shown previously to depend on the 3′UTR (Schutt et al., 2009).

Crosslinking-immunoprecipitation combined with high-throughput sequencing (HITS-CLIP) to generate an extensive list of direct FMRP targets from mouse brain *in vivo* (Darnell et al., 2011) . HITS-CLIP revealed that FMRP binds most frequently to the coding regions of mRNA as opposed to motifs within the 5′ and 3′ UTRs, supporting a ribosome stalling model of FMRP mediated repression. Many FMRP targets encode presynaptic as well as postsynaptic proteins. PAR-CLIP was also utilized as a method to identify direct FMRP targets in cultured cells overexpressing epitope tagged FMRP. Bioinformatic analysis indicated enrichment of ACUK and WGGA sequences as two potential RNA-recognition elements for FMRP, located more frequently in coding sequences but also within UTRs (Ascano et al., 2012). A bioinformatics comparison of Darnell and Ascano CLIP tags showed highly significant overlap and also validated the enrichment of the TGGA among other motifs (Anderson et al., 2016). Future work is need to investigate the overlapped data set and role of dysregulated translation of a defined set of mRNAs that may be central to the core pathology underlying FXS. Although there seem to be opposing views that FMRP interacts with coding and UTR sequences, it is likely that both mechanisms are utilized for different types of translational control or mediate other functions e.g. mRNA stability transport.

FMRP contains multiple structural motifs that allow it to bind directly to its RNA targets, such as KH domains and an RGG box (Ashley et al., 1993; Siomi et al., 1993). A single point mutation in *Fmr1*, the gene encoding FMRP, reveals the importance of FMRP's KH2 domain for RNA binding (De Boulle et al., 1993; Siomi et al., 1994). The missense mutation of an isoleucine 304 to asparagine (I304N) was discovered in a patient diagnosed with

fragile x syndrome despite lack of *FMR1* silencing and a sufficient production of FMRP. However, I304N FMRP does not associate with polyribosomes(Feng et al., 1997). The severe phenotype due to one missense mutation indicates that an essential function of FMRP relies on an intact KH2 domain. This suggests that loss of mRNA binding and/or polyribosome association is causative of FXS. Indeed, while wildtype FMRP is able to bind RNA, binding of FMRP I304N to RNA is impaired (Siomi et al., 1994). FMRP also contains an RGG box, a motif rich in arginines and glycines (Siomi et al., 1993). The RGG box facilitates the recognition and binding of G-quadruplexes to FMRP (Blackwell et al., 2010; Ramos et al., 2003). FMRP's multiple functional domains allow for various mechanisms for FMRP to recognize and bind mRNA. The function of RGG box binding to FMRP targets may therefore be important for mRNA localization (Stefanovic et al., 2015; Subramanian et al., 2011).

4. FMRP interacts with the RNA-Induced silencing complex

One of the ways that FMRP can regulate the translation of specific mRNAs is through the interaction with microRNAs and components of the RNA-Induced Silencing Complex (RISC), such as Argonaute1 and Argonaute2 (Jin et al., 2004; Li et al., 2014; Muddashetty et al., 2011). Specific miRNAs guide RISC to target mRNAs and repress their translation via mRNA degradation or prevention of translation initiation. FMRP binding to target mRNA contributes to the ability of the miRNA-RISC complex to recognize the target mRNA. For example, FMRP promotes the binding of a miR-125a-RISC complex on PSD-95 mRNA and repress its translation (Muddashetty et al., 2011). Additionally, FMRP promotes the miR-196a mediated translational repression of HoxB8, a mRNA that FMRP can directly bind (Li et al., 2014). As another example, FMRP cooperates with miR-125b to suppress translation of NR2A mRNA (Edbauer et al., 2010).

FMRP interaction with RISC alone is not sufficient to promote the miRNA-induced silencing of translation. Recent evidence indicates that post-translational modifications of FMRP are necessary to guide the miRNA-RISC complex to the target mRNA. Phosphorylated FMRP at serine 499 promotes the formation of a miR-125a-RISC complex on *PSD-95* mRNA to repress translation (Muddashetty et al., 2011). Downstream of mGlu5 signaling, FMRP is dephosphorylated by PP2A, as a switch to de-repress translation (Muddashetty et al., 2011; Narayanan et al., 2007). The dephosphorylation of FMRP disrupts the interaction of FMRP to Argonaute2 as well promotes the release of miR-125a-RISC from *PSD-95* mRNA (Muddashetty et al., 2011). Phosphorylation of FMRP as a translational repressive switch has also been observed for other miRNA-mRNA interactions. Phosphorylated FMRP also promotes miR-196a-induced translational repression of HoxB8 (Li et al., 2014). The phosphorylation/dephosphorylation of FMRP did not impact the ability of FMRP to bind directly to the target mRNA. In both of the mentioned studies, mGlu5 stimulation was utilized to modulate the phosphorylated state of FMRP. Furthermore, the ubiquitination proteasome system has been shown to further modify dephosphorylated FMRP to facilitate the translation of FMRP-target RNAs (Nalavadi et al., 2012).

Evidence suggests that multiple kinases may regulate FMRP phosphorylation. Ribosomal protein S6 kinase, S6K1, plays a role in activity-regulated phosphorylation of FMRP at

serine 499 (Narayanan et al., 2008). However, phosphorylation of FMRP at serine 499 is also modulated by Casein Kinase II (Bartley et al., 2014; Bartley et al., 2016). Secondary phosphorylation of FMRP at other serines is also regulated (Bartley et al., 2016). Despite the various models of FMRP phosphorylation, it has been well established that the phosphorylated state of FMRP is a mechanism for FMRP to recruit miRNA-dependent pathways to inhibit the translation of target mRNAs. Future studies are needed to better understand the role of multiple kinases in post-translational modifications of FMRP and how FMRP associates with RISC complex to regulate translation.

5. FMRP binds polyribosomes

FMRP has been well characterized to directly associate with polyribosomes (Corbin et al., 1997; Darnell et al., 2011; Stefani et al., 2004), suggesting another mode by which FMRP can directly regulate translation. It is currently thought that FMRP association reversibly stalls ribosomes (Darnell et al., 2011). FMRP-lacking mouse brains have an increased ribosome transit rate (Udagawa et al., 2013), suggesting that FMRP directly stalls ribosomes. Specific regions of FMRP interact with polyribosomes to affect translation. Exploitation of the I304N FMRP mutation (as described previously) has demonstrated the essential role of the KH2 domain in FMRP-polyribosome association (Darnell et al., 2005; Feng et al., 1997). Additionally, arginines within the RGG box are required for FMRP-polyribosome association (Blackwell et al., 2010). However, it is unclear whether the KH2 domain and RGG box utilize cooperative or separate mechanisms to associate to polyribosomes. Similar to how phosphorylation affects FMRP's association to RISC (Li et al., 2014; Muddashetty et al., 2011), phosphorylation can also regulate the downstream effects of FMRP-polyribosome interactions (Ceman et al., 2003). Both phosphorylated and unphosphorylated FMRP can bind polyribosomes, but it has been observed that primarily the phosphorylated form is associated with stalled polyribosomes (Ceman et al., 2003). Thus, dephosphorylation may play a key role in regulating the reversibility of FMRP-mediated translational suppression. A major unanswered question is how FMRP mediated ribosome stalling is directly regulated by physiological signals. Considering how FMRP phosphorylation affects FMRP's ability to interact with RISC (as discussed above) and polyribosomes, there is a possibility that these two pathways of translational regulation are tightly coordinated with one another. It is unclear if the translational phenotypes in FXS cells are primarily due to the loss of FMRP binding to polyribosomes or if other mechanisms discussed in this review are primary contributors to the underlying pathology.

FMRP's stalling of polyribosomes can be utilized to better understand pathology resulting from impaired translation in FXS models. Recent studies have utilized Translating Ribosome Affinity Purification (TRAP) to identify novel mechanisms by which FMRP mediates translation (Ouwenga et al., 2017; Thomson et al., 2017). Since FMRP-lacking neurons have an increased translation rate (Udagawa et al., 2013), TRAP following by RNA-sequencing can identify excessively translated transcripts due to FMRP loss. This method has explored understudied targets of FMRP, such as muscarinic acetylcholine receptor 4 mRNA (Ouwenga et al., 2017). Combination of the TRAP method with synaptoneurosomal fractionation demonstrates an enrichment of FMRP binding in mRNAs that are translated in dendrites (Thomson et al., 2017). This method provides a novel tool to observe how FMRP

may play distinct roles in different regions of the neuron and thus help to elucidate how FMRP regulates local translation.

6. Regulation of FMRP and *Fmr1* mRNA by association with other RNA-binding proteins

Elucidation of RNA binding proteins that interact with FMRP and *Fmr1* mRNA have given insight into potential mechanisms of FMRP-mediated translational regulation, as well as impairments in neurodevelopmental disorders other than FXS. Aside from interacting with protein components of RISC, FMRP interacts with other RNA-binding proteins to regulate translation. FMRP's direct association to MOV10, an RNA helicase, modulates the ability of RISC to bind to target mRNA (Kenny et al., 2014). FMRP can recruit MOV10 to unwind mRNA for association with RISC to lead to translational suppression (see Fig. 1). On other mRNAs, the FMRP-MOV10 interaction may prevent RISC association, which promotes translation (Kenny et al., 2014). Thus, FMRP's association with MOV10 is both cooperative and dynamic, leading to different consequences on translation for specific mRNAs.

New evidence in a Drosophila model demonstrates FMRP's interaction with the RNA-binding protein dNab2, the ortholog of human ZC3H14 (Bienkowski et al., 2017). ZC3H14 is a poly(A)-binding protein whose loss leads to an inherited form of intellectual disability (Pak et al., 2011). dNab2 and dFMRP interact in neurons in both the cytoplasm and nucleus (Bienkowski et al., 2017). Additionally, dNab2 directly binds to *CAMKIIα* mRNA, a well-characterized FMRP target (Darnell et al., 2011), and suppresses its translation (Bienkowski et al., 2017). Together, these findings reveal a new player linked to intellectual disability that is involved in the mechanism of FMRP mediated regulation of translation of specific mRNAs.

Investigation of a variant in the 3′UTR of the *FMR1* gene found in developmentally delayed patients without FXS revealed a novel interaction with the RNA-binding protein HuR (also known as ElavL1) (Suhl et al., 2015). HuR typically binds adenine/guanine rich elements in mRNAs and promotes mRNA stabilization (Brennan and Steitz, 2001). The inability of HuR to bind *Fmr1* mRNA in this patient leads to a loss of RNA stability, as demonstrated by a faster mRNA decay rate of mutated *Fmr1* mRNA compared to wildtype *Fmr1* (Suhl et al., 2015). Aside from the direct influence HuR binding has on *Fmr1* mRNA stability, the interaction is necessary for mGlu5-dependent protein synthesis in primary neurons which was impaired in mutated *Fmr1* 3′UTR (Suhl et al., 2015). Thus, the interaction between HuR and *Fmr1* mRNA has downstream consequences on translation and expression of FMRP.

7. Role of FMRP in local translation

Following the findings of impaired dendritic spine morphology in brain tissue of patients with FXS (Hinton et al., 1991; Irwin et al., 2001; Rudelli et al., 1985), much research was focused on understanding spine phenotypes. A hallmark phenotype has been the preponderance of long, thin and morphologically immature dendritic spines (Bagni and Greenough, 2005). Dendritic spines in *Fmr1* KO mice are also less stable. Using *in vivo*

time-lapse imaging in L2/3 cortex revealed a persistent increase in spine instability in *Fmr1* KO mice, suggestive of developmental delay (Cruz-Martin et al., 2010; Suresh and Dunaevsky, 2017). Similar results were also observed in the visual cortex (Nagaoka et al., 2016), and L5 pyramidal neurons of *Fmr1* KO mice revealed an increase in spine turnover (Pan et al., 2010). Moreover, activity dependent elongation of the early dendritic protrusion remained absent in *Fmr1* KO mice (Cruz-Martin et al., 2012), which is proposed to underlie the defect in spine stabilization and thus the persistent immature spine phenotype in FXS.

Since FMRP is a RNA binding protein (Ashley et al., 1993; Siomi et al., 1993) with an inhibitory effect on mRNA translation (Li et al., 2001), a possible way by which FMRP affects spine dynamics and spine morphology is by influencing local mRNA translation in dendrites, particularly because of the known presence of polyribosomes in dendrites (Steward and Levy, 1982). Impaired local translation is suggested as a mechanism of pathogenesis in FXS. FMRP is localized in RNA granules in dendrites and spines (Antar et al., 2006; Feng et al., 1997) as well as in developing axons in culture (Antar et al., 2004) and mature axons *in vivo* (Akins et al., 2009). FMRP is normally associated with the translational machinery (Corbin et al., 1997; Feng et al., 1997; Siomi et al., 1996; Tamanini et al., 1996). FMRP interacts with a number of proteins among which are: its homologues FXR1 and FXR2 (Zhang et al., 1995), nucleolin (Ceman et al., 1999), NUFIP (Bardoni et al., 1999) and CYFP1/2 (Napoli et al., 2008; Schenck et al., 2003). In addition, FMRP granules interact with kinesin to function in the transport of some mRNAs (Dictenberg et al., 2008). Thus, FMRP has all the characteristics necessary to support its role as major regulator of local translation in both pre- and post-synaptic terminals. Further, most of the studies studying the role of FMRP in mRNA translation regulation have been dedicated to the postsynaptic compartment. However, there is increasing literature indicating a role of FMRP in regulating axonal mRNA translation, in certain brain circuits both during synaptogenesis and in adult life. Moreover, a role for FMRP in regulating mRNA translation during neuronal differentiation and neurogenesis was established.

8. Postsynaptic mRNA translation regulation

The case for local translation in neurons was gaining traction following the identification of polyribosomes in dendrites and at spines (Steward and Levy, 1982) and the mounting evidence of local protein synthesis in neurons (Rao and Steward, 1991; Torre and Steward, 1992). Despite the numerous lines of evidence suggesting the role of FMRP in regulating local translation in dendrites, there was little direct evidence owing to lack of suitable methods. In a seminal study by (Aakalu et al., 2001) using a GFP reporter under control of the 3′UTR of CaMKIIα, a target of FMRP, local translation in isolated dendrites was directly visualized. This study started the quest for developing new methods that would allow to assignment of protein synthesis to specific dendritic sub-domains. Strong synaptic stimulation has been shown to determine the translocation of the polyribosomes from the base of the spine into the spine head (Ostroff et al., 2002), raising the possibility that some translation occurs in the spine head. However, no direct method allowed for visualization of mRNA translation with enough spatial resolution to resolve this.

A study using microinjection of fluorescently labeled mRNA coding for Venus fused Arc or FMRP aimed to visualize the translational output of mRNA granules in dendrites (Tatavarty et al., 2011). They also found that newly synthesized Venus-Arc and Venus-FMRP molecules are clustered near mRNA granules and are detected both at the base of the spine and spine head which increased rapidly by dihydroxyphenylglycine (DHPG) stimulation in less than a minute. Single molecule imaging of Venus-Arc and Venus-FMRP allowed the visualization of translation with high spatial and temporal resolution. FMRP synthesis in a synaptosome preparation previously suggested that FMRP itself may be locally synthesized (Angenstein et al., 1998). Consistent with this idea, *Fmr1* mRNA is localized in dendrites (Antar et al., 2004). A different study visualized *Arc* mRNA local translation using the bioluminescence of Gaussia luciferase detected increased translation at 15 s after stimulation with glutamate, thus confirming the rapid kinetics of activity regulated Arc mRNA translation (Na et al., 2016). Another study using a Venus based reporter visualized the local translation of *PSD-95* mRNA, a target of FMRP (Muddashetty et al., 2007), in mouse hippocampal neurons. Single molecule imaging of *PSD-95* mRNA translation in WT and *Fmr1 KO* neurons revealed that translation occurs at the base of the spine and in spine head near the synapse and that basal translation was increased and activity regulated translation was occluded in *Fmr1 KO* neurons (Ifrim et al., 2015). These methods for visualizing local translation in neurons have limitations regarding the number of molecular species that can be visualized at once. Given the numerous mRNA targets of FMRP localized at the synapse it would be useful to be able to visualize dynamically a multitude of the newly synthesized proteins in the dendrites with high spatial and temporal resolution in order to gain a more complete understanding of the role of FMRP in modulating protein homeostasis at the synapse.

9. Presynaptic mRNA translation regulation

In primary neuronal cultures, FMRP granules localize in the growth cone and in the distal segment of axons (Antar et al., 2006). In addition, *Map1b* mRNA, a target of FMRP, was colocalized in the growth cone. Moreover, in *Fmr1 KO* neurons the motility of growth cone was reduced, thus establishing a role for FMRP in axons prior to synaptogenesis, and suggesting a possible role of axonal defects in FXS pathogenesis.

In a series of elegant publications from Justin Fallon and Michael Akins, distinct types of FMRP granules in axons, termed fragile x granules (FXGs) were identified and characterized. FXGs in mouse neurons are located in the axons of a subset of brain regions including the frontal cortex, CA3 area of the hippocampus and the olfactory bulb glomeruli (Christie et al., 2009). FXGs have been classified into four types based on their composition. FXGs can contain FMRP and both its homologs, FXR1 and FXR2 (type I), FMRP and FXR2 only (type II), FXR1 and FXR2 only (type III), or only FXR2 (type IV) (Chyung et al., 2018), with all types containing FXR2. FXR2 has been shown to be necessary for FMRP recruitment to FXGs (Christie et al., 2009). The localization of FXR2, and consequently FMRP, to the axon is controlled by FXR2 N-myristoylation on glycine 2 (Stackpole et al., 2014). While FMRP is not required for FXGs formation, it does suppress the number of FXGs (Christie et al., 2009). FXGs in axons colocalize with both ribosomal subunits, but FXR2, FMRP and the ribosomes are enriched in distinct subdomains of the ~ 200 nm FXGs

(Akins et al., 2017). FXGs also associate with polyadenylated RNA, and, interestingly, with a small subset of FMRP mRNA targets out of 21 targets tested: *Ctnnb1*, beta-catenin), *Map1b, Ncam1*, and *OMP*, while other FMRP targets were not present (*DLG5, PSD-95*), *Fmr1* or Bassoon for example). Absence of FMRP or FXR2 does not impair axonal localization of the ribosomes or of polyadenylated RNA, suggesting that the axonal localization of the ribosomes and polyadenylated RNA is independent of FMRP and FXR2 (Akins et al., 2017).

Interestingly, some identified FXGs contained two types of mRNA: *Ctnnb1*, coding for beta catenin, and APC, coding for APC (Adenoma polyposis coli). Beta-catenin has a role in regulating the distribution of the pool of presynaptic vesicles (see Fig. 1). APC has a role as a negative regulator of beta catenin. Thus, both messages can be present in the same FXGs, and are functioning in the same pathway (Chyung et al., 2018). Thus, one type of FXGs might coordinate the expression of multiple proteins functioning in the same pathway, as it has been previously proposed (Carson et al., 2008). In mouse, FXGs were developmentally regulated with FXGs number, decreasing significantly between P15 and P60 in all brain regions studied except for olfactory glomeruli. In the olfactory bulb, Olfactory Sensory Neurons (OSN) have a high turnover rate and thus the presence of FXGs in this area could be linked to the new generated neurons forming synapses. Consistent with this model, in a paradigm in which OSN were ablated, FXGs number dropped initially, followed by recovery and even an “overshoot” during regeneration, when new OSN were generated and formed synapses (Christie et al., 2009). In contrast to mice, rat FXGs persist in adult life in hippocampus and frontal cortex, in addition to olfactory bulb. Moreover, FXGs have been found also in human hippocampus in individuals as old as 57 years, pointing to a role for FMRP in regulating (synaptic plasticity related) axonal mRNA translation in the adult nervous system (Akins et al., 2017). FXGs display a wide heterogeneity given by their protein composition (which could be further amplified if the isoforms are considered), the mRNA composition, and the specific circuit in which they are expressed. Therefore, FXGs could imprint a wide variety of modulation on axonal biology (including synaptic strength) in a circuit specific manner. There are several questions that still await answers. For example: What determines the specific type of FXGs expressed in a specific brain circuit, what determines the mRNA species associated with the FXGs and what are the mechanisms involved in FXGs associated mRNA localization in axons?

10. Glia in fragile X syndrome

Glial cells are critical for shaping the synaptic environment, including local structural and functional modification of individual synapses. Loss of astroglial FMRP can downregulate mGluR5 receptors in a translation dependent manner (Higashimori et al., 2013) and their selective deletion contributes to fragile x syndrome phenotypes in *Fmr1* KO mice (Higashimori et al., 2016). Although the effect of loss of FMRP on translation machinery in glial cells needs to be explored further, their importance in FXS pathogenesis is underscored by the fact that neuron co-cultures with *Fmr1* KO astrocytes lead to a delay in synaptogenesis as well as increased dendritic branching at 7 days *in vitro* (Jacobs and Doering, 2010). Further, astrocyte-specific *Fmr1* KO mice displayed defects in spine density and dynamics as well as behavioral deficits. Expression of FMRP only in astrocytes could

not rescue the spine or behavioral defects of *Fmr1* KO mice pointing to both neuronal and astrocyte contributions to FXS pathogenesis (Hodges et al., 2017). *Fmr1* KO astrocytes displayed increased expression of Thrombospondin-1 (TSP-1), an astrocyte-secreted protein, involved in the regulation of spine development and synaptogenesis (Cheng et al., 2016). On the other hand, Nestin mRNA, encoding the intermediate filament protein Nestin, has been shown to be localized to astrocyte protrusions in a FMRP and FXR1 dependent manner, suggesting possible defects in the regulation of Nestin expression in FXS (Thomsen et al., 2013). Thus, loss of FMRP in astrocytes is involved in FXS pathogenesis, possibly by altering gene expression in astrocytes with effects on synaptogenesis.

A different but significant aspect of glial function involves the process of myelination, which is perturbed in a subpopulation of FXS patients (Barnea-Goraly et al., 2003; Haas et al., 2009; Hoeft et al., 2010). FMRP has been shown to regulate myelin binding protein (MBP) in developing oligodendroglia (Wang et al., 2004), a protein essential for the formation of compact myelin sheaths around axons. Lack of FMRP prevents inhibition of MBP mRNA translation (Li et al., 2001), with MBP levels consequently seem to be increased in FXS. However, studies in *Fmr1* KO mouse oligodendrocytes did not find differences in the expression of myelin proteins, suggesting that the rodent models may not recapitulate the myelin abnormalities observed in FXS (Giampetruzzi et al., 2011). However, another study found region specific delayed myelination specially during the first postnatal week (Pacey et al., 2013). Nevertheless, with increasing evidence indicating glial defects in fragile x syndrome, researchers may need to look beyond neurons to find potential therapeutic interventions.mRNA translation regulation during neurogenesis

Although the majority of RNA dysregulation caused by absence of FMRP has been studied in mature neuronal populations, it is important to consider the neuroanatomical and neurogenesis defects observed in the brain of FXS patients (reviewed in (Castren, 2016), which suggests a critical role played by FMRP during developmental stages. Surprisingly, little is known about FMRP's role in regulating mRNA transport and translation during neurogenesis. Radial glia cells (RGC) are the neuronal progenitors in the developing brain. RGC have a basal process (along which newly formed neurons migrate to form the cortical layers) that extends from near the ventricle to the basal lamina situated under meninges, where it forms a basal endfeet. A recent exciting study has shown that FMRP granules and mRNA granules are actively transported along the basal process. In the basal end feet of RGC, FMRP is bound to a specific subset of FMRP mRNA targets, encoding for signaling and cytoskeletal regulators, which are locally translated, among which are: CyclinD2 (*Ccnd2*), *Vash1, Ptpn11, Apc*, and *Kif26a*, mRNAs. Among the targets identified, a high proportion is associated with autism suggesting that an impairment in neurogenesis due to loss of mRNA translation regulation in FXS could be involved in autism in FXS (Pilaz et al., 2016). In addition, RNA immunoprecipitation and microarray analysis of endfeet indicated a role of FMRP in regulation of transport and localization of *Kif26a* mRNA (Pilaz et al., 2016), and revealed the local transcriptome in radial glia. To summarize, further understanding of mRNA dysregulation in FXS during neurogenesis can provide a new mechanistic dimension on FMRP mediated local translation and identify potential new targets for therapeutic intervention.

11. Abnormal protein synthesis in FXS animal models

Based on the understanding of FMRP biology and how it can regulate translation both locally and globally, it is imperative that loss of FMRP in FXS will consequently cause dysregulated protein synthesis. This hypothesis has now been supported and verified by several bodies of research (Osterweil et al., 2010; Qin et al., 2013). While *Fmr1* KO mice has been the most widely used model to understand pathophysiology in FXS, researchers have also expanded their investigation using *Fmr1* KO rats for better comparison of phenotypes that results from FMRP deletion as well as bridge gaps between mouse and human findings (Hamilton et al., 2014; Tian et al., 2017; Till et al., 2015). Abnormal protein synthesis can affect a wide spectrum of phenotypes in FXS via either affecting neuronal signaling pathways functionally or abnormal dendritic spines morphologically. Therefore, therapeutic strategies in the last decade have been primarily focused on addressing one of the above phenotypes.

Among the several neuronal signaling pathways implicated in FXS, mGluR pathway is probably the most extensively studied, eventually leading to the mGluR theory which states that absence of FMRP results in exaggerated mGlu5- mediated protein synthesis, dysregulated signaling and aberrant synaptic plasticity (Bear et al., 2004). This was consistent with the large body of findings which indicated that excessive glutamate signaling which prevails in FXS underlie several of the clinical manifestations (Bear et al., 2004). However, following the negative outcome of mGlu5 antagonists in clinical trials (Mullard, 2015), significant focus of current FXS research has shifted to find alternative therapeutic targets by identifying mRNAs regulated by FMRP located downstream to mGluR and essential for normal dendritic spine morphology and synaptic function.

12. Targeting protein synthesis affecting synaptic plasticity in FXS animal models

FMRP targets downstream of mGluR signaling pathway plays multifaceted roles – this includes molecules playing both structural and functional roles during protein synthesis, located both upstream and downstream of translation. Such targets and their role in FXS have been discussed at length in a review by Richter, Bassell and Klann (Richter et al., 2015). This section will focus on some of the FMRP targets which not only plays a critical role in FXS pathophysiology but also could be rescued via both genetic and pharmacological manipulation thereby emerging as significant targets for therapeutic intervention in FXS (*see* Table 1). Stimulation of group 1 metabotropic receptors results in activation of PI3K-mTOR and ERK pathways which together forms a critical nexus in regulating the protein synthesis machinery. FMRP directly controls translation and synthesis of two key regulatory components of PI3K signaling complex – p110β subunit and the PI3K enhancer (PIKE), which displays exaggerated basal activity and reduced mGluR dependent activity in FXS mice and human FXS cells (Gross et al., 2010; Gross and Bassell, 2012). Recent studies found that genetic reduction of p110β and PIKE activity in mice and *Drosophila* abolished excessive PI3K signaling and protein synthesis, thereby providing a potential alternative strategy to alleviate FXS associated phenotypes including abnormal synaptic plasticity,

impaired spine morphology and behavioral abnormalities (Gross et al., 2015a,b). It is important to note that targeting specific components of PI3K signaling pathway rather than global reduction is desirable as the overall signaling pathway remains intact which is essential for various other cellular functions (Hawkins and Stephens, 2015; Stark et al., 2015; Vyas and Vohora, 2017). Following the same line, a recent investigation by Gross et al. (under revision) tested a novel class of p110β subunit selective inhibitors (GSK-6A), in collaboration with GlaxoSmithKline (GSK), which rescued many FXS-associated molecular and behavior phenotypes in *Fmr1 KO* mice, thereby showing significant promise as a potential therapeutic intervention in future FXS research.

A different strategy to manipulate the protein synthesis machinery is to target translation control molecules. P70S6Kinase1 (S6K1) is a key translation initiation and elongation factor. It is also a substrate for mTORC1 that phosphorylates a diverse set of substrates including the initiation factor eIF4B. Enhanced S6K1 signaling in *Fmr1 KO* mice can cause multiple FXS associated behavioral and molecular phenotypes including exaggerated protein synthesis, inappropriate social behavior, behavioral inflexibility, altered dendritic spine morphology, audiogenic seizures and macroorchidism (Narayanan et al., 2008). Several of the above-mentioned phenotypes, including increased protein synthesis, could be reduced to wildtype levels with both genetic reduction and pharmacological inhibition of S6K1, suggesting S6K1 as an attractive target in FXS treatment (Bhattacharya et al., 2012; Bhattacharya et al., 2016; Sawicka et al., 2016).

The protein translation machinery in FXS can be further targeted by regulating translation initiation through its interaction with the cap binding translation initiation factor, eIF4E, which binds with eIF4E binding proteins (4E-BP) to repress translation. Recently, exaggerated protein synthesis has been als associated with increased interaction of eIF4E to eIF4G (Santini et al., 2017). This study further found that blocking the interaction pharmacologically using 4EG1 alleviated cognitive, spine abnormalities as well as exaggerated mGluR-LTD in FXS mice.

Another validated FMRP candidate acting downstream is Striatal- Enriched-protein Tyrosine Phosphatase (STEP). STEP promotes weakening of synapses by dephosphorylating its substrates, including ERK1/2 and subunits of N-methyl-d-aspartate (NMDA) and AMPA receptors. However, FMRP regulated translation and mGluR-dependent STEP synthesis is absent in *Fmr1* KO mice, thereby causing FXS associated phenotypes. Genetic reduction/ elimination of STEP as well as more recent pharmacological inhibition has reversed audiogenic seizures, hyperactivity and anxiety stress while enhancing sociability in *Fmr1 KO* mice (Chatterjee et al., 2018; Goebel-Goody et al., 2012). Therefore, it is essential to further scrutinize the downstream components of mGluR signaling and its impact on the enhanced protein synthesis observed in FXS as a possible future therapeutic avenue to pursue.

13. Targeting aberrant protein synthesis affecting dendritic spine morphology in FXS animal models

Locally translated proteins are important for dendritic spine morphology. Specific proteins and biochemical pathways have been associated with spine morphogenesis (Tada and Sheng, 2006) many of which affect actin dynamics in spines. Rho GTPases such as Rac1, for example, are upstream modulators of actin polymerization. Actin is enriched in postsynaptic dendritic spines and plays a pivotal role in spine morphogenesis and synaptic plasticity. Depolymerization of actin by cofilin limits spine density and maturation. One of the most striking phenotype in FXS is abnormal dendritic spine morphology, which has been attributed to the absence of FMRP and its effect on protein synthesis, signaling and actin spine dynamics (He and Portera-Cailliau, 2013; Irwin et al., 2001; Pan et al., 2010). Lack of FMRP and its consequent effect on the translational machinery leads to elevated Rac1-PAK1 signaling and aberrant actin dynamics therefore causing abnormal dendritic spines as well as impaired synaptic transmission in the somatosensory cortex of FXS mice. In fact, PAK or Rac1 activity when inhibited by small molecule inhibitors rescued altered glutamatergic signaling, impaired sensory processing and abnormal fear conditioning in FXS mice (Dolan et al., 2013; Martinez and Tejada-Simon, 2017; Pyronneau et al., 2017).

Alternatively, the process of protein synthesis, which impacts the cytoskeletal machinery, can be regulated at different steps and targeted for therapeutic intervention. A recent study in a FXS mouse model revealed that absence of FMRP apart from exaggerated Rac1 activity can also affect the dendritic spine dynamics by differential eIF4E binding. This disrupts the equilibrium between actin dynamics and protein synthesis, leading to aberrant spine morphogenesis and brain function in FXS. Interestingly, pharmacological inhibition of the eIF4E-eIF4G interaction with 4EGI-1 reduced the over activation of Rac1-PAK-cofillin signaling in FXS mice. A new possible avenue therefore is to use drugs that target either or both pathways to balance protein synthesis and actin dynamics, thereby counteracting abnormal phenotypes in FXS (Santini et al., 2017).

Another potential therapeutic avenue is to regulate the environment of the extracellular matrix and the underlying cytoskeletal machinery by targeting matrix metalloproteinase 9 (MMP9) which plays a critical role in spine morphology, synaptic plasticity, learning and memory and has been identified as a FMRP target. Translation of *Mmp-9* mRNA is locally regulated by FMRP in dendrites following neuronal stimulation (Dziembowska and Wlodarczyk, 2012; Janusz et al., 2013). In addition, translation of *Mmp9* mRNA and several additional members of the family, which are regulated by eIF4E phosphorylation, are enhanced in FXS post mortem brains. Higher MMP-9 activity, which is in part attributed to increased eIF4E phosphorylation and enhanced mTORC1/ERK signaling, has been previously reported in *Fmr1 KO* mouse brains, suggesting that MMP-9 dysregulation may contribute to FXS-associated deficits (Gkogkas et al., 2014; Reinhard et al., 2015). In fact, genetically reducing MMP9 rescued FXS associated phenotypes including audiogenic seizures in FXS mice model (Lovelace et al., 2016). Apart from MMP9, other family members such as MMP1 but not MMP2 has also been recently shown to be important for activity dependent synapse development in a fly model of FXS (Dear et al., 2017).

Metformin – a first line therapy and a FDA approved drug to treat type II diabetes was recently tested in FXS mice model, due to its ability to inhibit the mTORC1 and ERK pathways. Chronic administration of metformin to adult *Fmr1* KO mice for 10 days significantly improved core FXS phenotypes including social behavior, self-grooming, enhanced LTD and abnormal dendritic spine morphology (Gantois et al., 2017). Although the ERK inhibitor lovastatin has previously been shown to ameliorate some aspects of the FXS phenotype in mice (Osterweil et al., 2013), metformin has rescued a wide variety of FXS associated symptoms and therefore should be further investigated as a potential therapeutic intervention to alleviate the aberrant protein synthesis.

14. Modeling *Fmr1* silencing and CGG repeat expansion in FXS hPSCs

Over the last two decades animal models have provided us with tremendous insight into the biology of FMRP and the potential mechanisms underlying FXS. Highly robust and promising preclinical findings in the *Fmr1* KO mouse model demonstrating the effectiveness of group 1 metabotropic receptor antagonists in the treatment of FXS led to several large clinical trials, however these trials failed to meet their primary endpoints. While this could be attributed to the need for improved trial design and better-defined outcome measures and clinical endpoints, a key concern is that although the FXS knockout mouse allows us to investigate loss of function of FMRP, it does not capture critical features of the human condition such as the expansion of the trinucleotide repeat, and the mechanism of methylation and silencing of the *FMR1* gene.

Our knowledge of FXS in humans has thus far largely been limited to postmortem studies of the brain. However, recent advances in neuronal differentiation using human pluripotent stem cells (hPSCs), including embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), have provided us with a biological model system that allows study of FXS in a disease relevant cell type. Importantly, hPSCs allow investigation of molecular events underlying the CGG repeat expansion and epigenetic silencing of *FMR1* in a developmentally regulated manner. In the first characterized FXS hESC line (Eiges et al., 2007) and in chorionic villi samples from FXS fetuses (Willemsen et al., 2002), it was found that despite the presence of the full mutation of over 200 CGG repeats, the *FMR1 gene* is transcribed and the promoter region is unmethylated. A subsequent study from the same group, however, showed that some FXS hESCs may be abnormally methylated, and consequently do not express *FMR1* mRNA (Avitzour et al., 2014). Neuronal differentiation of FXS hESCs has also been implicated in the silencing of the gene (Colak et al., 2014; Telias et al., 2013; Urbach et al., 2010). One proposed mechanism for the epigenetic silencing of the *FMR1* locus is that *FMR1* mRNA transcripts interact with the expanded CGG repeat region forming RNA-DNA duplexes which in turn mediate *FMR1* silencing (Colak et al., 2014). In contrast to FXS hESCs, induced pluripotent stem cells (hiPSCs) generated from FXS patient fibroblasts are consistently hypermethylated, and lack *Fmr1* mRNA as well as the protein product FMRP, suggesting that the repressive epigenetic marks of the original somatic cells are retained during the reprograming process (Avitzour et al., 2014; Sheridan et al., 2011; Urbach et al., 2010). Interestingly, iPSCs generated from the fibroblasts of male patients with an unmethylated full mutation acquire the hypermethylation and subsequent transcriptional silencing of *FMR1* (de Esch et al., 2011) . In another study,

researchers aimed to investigate whether the hypermethylation and silencing of *FMR1* was dependent on the maintenance of the CGG repeat expansion. To this end, they employed a CRISPR/Cas9-mediated strategy to correct the expanded CGG repeat and showed that this resulted in demethylation of *FMR1* as well as restoration of *FMR1* mRNA in iPSCs as well as neural progenitor cells and neurons (Park et al., 2015). FXS hPSC models have given us an unprecedented opportunity to study mechanisms of *FMR1* silencing as well as CGG repeat expansion, ultimately paving the way for more accurately designed therapeutics.

15. Modeling cellular and molecular deficits in FXS hPSCs

Several studies have reported that the loss of FMRP results in morphological defects, dysregulated signaling and abnormal protein synthesis in *Fmr1* KO mouse neurons. Many of these core deficits have been shown to be rescued in the FXS mouse model by using genetic or pharmacological approaches that target key signaling pathways or cell surface receptors as described above. However these strategies have not translated to effective therapeutic approaches for human patients yet. The ability to reprogram somatic cells into iPSCs presents an unprecedented opportunity to investigate the consequences of this loss of function mutation in a patient-specific, disease-relevant manner. Furthermore, advances in neuronal differentiation methods have opened up the possibility of large-scale, high-throughput drug screens that can be used to identify and test the efficacy of compounds to treat FXS. In one of the earliest studies to use hPSCs to model FXS, the researchers generated clonal iPSC lines from a patient with mosaic expression of the mutation (possessing both premutation and full mutation alleles). They found that neurons differentiated from iPSC clones with the methylated full mutation did not express FMRP and exhibited deficits in neuronal morphology and differentiation as compared to FMRP-expressing neurons that were generated from iPSC clones with the unmethylated allele (Sheridan et al., 2011). In another study, FXS patient iPSC derived forebrain neurons were found to have fewer and shorter neurites, as well as defects in neurite initiation and extension (Doers et al., 2014). In contrast, studies from fetal cortex derived hNPCs (Bhattacharyya et al., 2008) and FXS hESC derived neurons(Telias et al., 2013) did not find any significant differences in neurogenesis and neurite formation compared to controls, although there appeared to be increased gliogenesis in FXS hESC derived neuronal cultures. This study further examined electrophysiological properties of these neurons and found that loss of FMRP in human neurons resulted in reduced frequency, amplitude of action potentials and poor synaptic formation (Telias et al., 2013). The same group conducted a more extensive morphological and functional analysis of FXS hESC derived neurons and found that FXS neurons had shorter and less tortuous neurites compared to controls, as well as smaller somata (Telias et al., 2015). These neurons also exhibited deficits in action potential firing, spontaneous synaptic activity and response to GABA (Telias et al., 2016) suggesting an overall immature phenotype that is in line with findings from the *Fmr1* KO mice. The work being done using hPSCs to model FXS in neurons is exciting and provides critical preclinical validation of work done in the knockout mouse, as well as allowing for the investigation of questions that thus far were impossible to answer in other biological systems. A major obstacle in working with hPSCs is the variability in results and lack of consistency in methods, which although appreciated when working with human samples,

needs to be optimized and standardized further across labs in order to help progress research in the field, and allow for more consistent, reproducible results.

16. Discussion and future perspectives

Currently, there are no proven effective treatments available for fragile x syndrome. Significant efforts from the fragile x scientific community has led to several successes in preclinical FXS models, however, a critical gap remains between basic and translational research complicating our path to finding effective therapeutic intervention for FXS. It is therefore essential to revisit the basics. This includes expanding our understanding of FMRP biology, its role in RNA translation, the various signaling pathways that are affected by loss of FMRP in FXS as well as investigating potential new preclinical models to better elucidate the mechanism underlying pathophysiology of FXS and identify new drug targets. A comprehensive review on current drug development and recent clinical trials in FXS can be found in reviews published recently (Berry-Kravis et al., 2017; Hagerman et al., 2017).

Research in the past decade has time and again found a critical role of mGluR5 signaling pathway in FXS, with exaggerated protein synthesis as a possible common underlying pathophysiologic mechanism. Therefore, targeting various downstream molecules of this key signaling pathway, which is also used by other receptor systems playing a role in protein synthesis, synaptic plasticity and dendritic spine morphology seems to be a logical and promising path to follow. Therefore, in this review, we focused on the new emerging roles of FMRP in FXS from a mechanistic point of view. The wide spectrum of direct and indirect mechanisms by which FMRP can regulate mRNA translation, both at presynaptic and postsynaptic level will shed new light on how its absence can impact neuronal functioning in FXS. Further, it is imperative to identify and explore preclinical models with underlying FMRP associated mechanistic targets affecting protein synthesis and dendritic spine morphology in general. Therefore, dysregulated FMRP targets, playing a mechanistic role in FXS, which have been rescued by both genetic and pharmacological interventions in preclinical models, hold unrealized potential as a therapeutic intervention in FXS and thereby needs to be further investigated for potential future clinical trials. At the same time, novel tools such as human pluripotent stem cell derived neurons holds great promise for the understanding of the molecular underpinnings of FXS and testing therapeutic strategies. The ease of differentiating hPSCs into neural progenitor cells and neurons has paved the way for high-throughput drug screens in FXS (Kaufmann et al., 2015; Kumari et al., 2015), which will be of critical importance in the development of treatment strategies for FXS.

This current review has discussed the recent developments pertaining to the understanding of inherent role of FMRP in mRNA translation, aberrant mRNA translation in FXS and associated dysfunctional glutamatergic signaling. The role of FMRP protein in FXS is diverse and extends well beyond glutamatergic signaling and protein synthesis This includes several non-canonical functions of FMRP, including its role in epigenetic regulation (Korb et al., 2017), as well as its important involvement in GABAergic signaling (Willemsen and Kooy, 2017). Further, nuclear FMRP, which binds to chromatin to modulate DNA damage response, therefore affecting chromatin stability in the process can have important implication in the pathophysiology of FXS (Alpatov et al., 2014). Therefore, it is evident

that FMRP plays a diverse set of roles and its loss can give rise to a wide spectrum of phenotypes as observed in FXS. It is therefore essential to explore and investigate new dimensions and understand the disease mechanism from multiple angles with the common goal of finding a treatment for fragile x syndrome.

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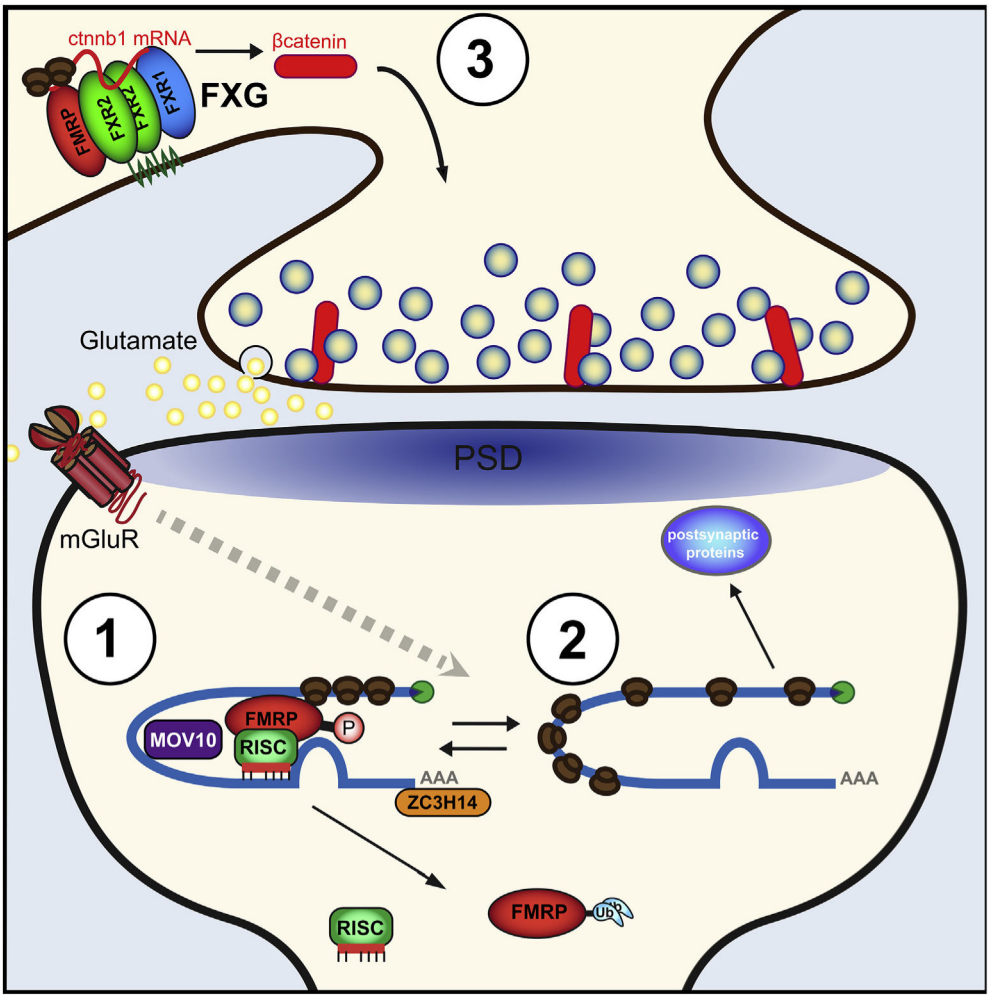

Fig. 1.

The schematic depicts multiple mechanisms by which FMRP regulates translation. (1) Phosphorylated-FMRP regulates translation of target mRNAs by directly associating with polyribosomes and mRNP complexes. FMRP associates with other RNA binding proteins such as MOV10 (RNA Helicase) and ZC3H14 to regulate translation. (2) FMRP can regulate mGlu5 mediated translation by dephosphorylation of FMRP, ubiquitination of FMRP and release of RISC complex. (3) Beta-catenin, a component of Fragile X granule, regulates the distribution of the pool of presynaptic vesicles and regulates plasticity. For the purpose of simplicity, this schematic does not represent the exhaustive list of FMRP targets that has been discussed in the review.

Table 1

FMRP Targets with Genetic and Pharmacological Rescue in Fragile X mice models.

		Genetic		Pharmacological		References
FMRP Target	**Function**	**Model**	**Phenotype Rescued**	**Compound**	**Phenotype Rescued**	
p110β	Component of PI3K signaling complex regulating translation	$p110\beta^{+/-}$	•Protein synthesis •Nest building •Repetitive behavior •Executive function	p110b subunit selective inhibitor (GSK-6A)	•Protein synthesis •Dendritic spines •Social behavior •Anxiety •Executive function	Gross et al. 2015a,b Gross et al. (under revision)
S6K1	Translation elongation factor	$S6K1^{-/-}$	•Protein synthesis •Dendritic spines •mGluR5-LTD •Social behavior •Behavioral flexibility	S6K1 inhibitors (PF-4708671 & FS-115) RSK inhibitor (BI-D1870)	•Protein synthesis •Dendritic spine •Audiogenic seizures •Social Novelty •Repetitive behavior	Bhattacharya et al. 2012 Bhattacharya et al. 2016
eIF4E	Translation initiation factor	NA	NA	4EG1	•Dendritic spine •mGluR5-LTD •Social behavior •Repetitive behavior	Santini et al. 2013 Santini et al. 2017
Striatal- Enriched-protein Tyrosine Phosphatase (STEP)	Enzyme promoting weakening of synapses by inhibiting ERK pathway	$STEP^{+/-}$ $STEP^{-/-}$	•Protein synthesis •Audiogenic seizures •Social behavior •Non-social anxiety	Small-molecule STEP inhibitor (TC2153)	•Protein synthesis •Dendritic spine •mGluR5-dependent LTD •Audiogenic seizures •Hyperactivity •Anxiety •Social behavior	Goebel-Goody et al. 2012 Chatterjee et al. 2018
MMP9	Enzyme involved in degradation of extracellular matrix	$MMP9^{-/-}$	•Protein synthesis •mGluR5-LTD •Auditory Processing •Anxiety •Social behavior	Cercosporamide, Metformin	•Protein synthesis •Dendritic spine •mGluR5-LTD •Audiogenic seizures •Anxiety •Social behavior •Repetitive behavior	Sidhu et al. 2014 Gkogkas et al. 2014 Gantois et al. 2017