Fragile X Syndrome: A Disorder of Synaptic Protein Synthesis Dynamics

Ravi S. Muddashetty*, Vijayalaxmi C. Nalavadi and Gary J. Bassell

Abstract | Fragile X syndrome (FXS) is a developmental disorder resulting from trinucleotide repeat expansion in the 5'UTR of FMR1 gene. Cognitive deficiency and autistic features are among the common phenotypes of FXS. FMR1 gene codes for the protein FMRP, the absence of which leads to abnormal dendritic spine morphology and defective synaptic plasticity in animal models of fragile X syndrome. FMRP is a selective RNA binding protein and is shown to interact with a large number of mRNAs and micro-RNAs. FMRP modulates the translation of a subset of dendritic mRNAs in response to neuronal activity and plays a critical role in synaptic plasticity. Several models have been proposed to explain the mode of FMRP interaction with target RNAs and mechanism of FMRP mediated translation regulation, while a comprehensive understanding of its function is still elusive. FMRP is also proposed to have a pivotal role in neuronal stem cell maintenance, neurogenesis and neuronal differentiation. The research on FMRP function has unearthed huge information about the intricate regulatory processes at the synapse and has highlighted importance of activity mediated translation in neurons. Progress in understanding the function of FMRP has helped to design targeted therapeutic approach for FXS and is leading the way for potential therapies for other autism spectrum disorders.

Genetic and environmental factors interact in complex ways to orchestrate the development of the nervous system. Differentiation and maturation of neurons, initial formation of neuronal network and equipping the network for plasticity are all largely determined by the interactions between gene expression and external stimuli. Defects in genetic information can adversely impact development resulting in severe defects in behavior, cognition and quality of life in the affected individual. Understanding the role of a single gene and how it regulates the expression of other genes and biological networks has provided important insight into how synapses develop in the brain. Fragile X syndrome (FXS), a single gene disorder, resulting from the loss of function mutation in the FMR1 gene, results in severe deficiency of cognition and behavioral abnormalities. The FMR1 gene is located on the X-chromosome and due to X-linked inheritance it is more prevalent in males (1:4000) than females (1:8000).\(^1\) Apart from cognitive deficiency and low IQ, patients with FXS have characteristic behavioral hyperactivity, impulsivity, attention problem, mood instability and anxiety. In males, physical features include loose connective tissue, prominent ears, flat feet, long face and macroorchidism in their adulthood. In females, many of the phenotypes are moderate including IQ deficiency.\(^2\) There is strong association between FXS and autism, one of the most common developmental disorders. Prevalence of autism among the individuals with FXS is approximately 18–36%,\(^3\) while more than 50% are reported to have some features of autism or autism spectrum disorders (ASD).\(^4\) Autism is, in fact, a group of disorders and the fragile X mutation is the leading cause of Down syndrome. It is a complex condition with no cure yet, and treatments are often only symptomatic. The research on FMRP function has led to the discovery of new therapeutic approaches for FXS and other autism spectrum disorders.

Loss of function mutation: It is a mutation resulting in less or no function of the gene product.

X linked inheritance: Inheritance of the genes linked to X-chromosome. In humans females have XX chromosome while males have XY. Thus X linked inheritance is more common in males, in contrast to the inheritance of traits on autosomal chromosomes where both sexes have same probability of inheritance.

Autism: Autism is a developmental disorder characterized by impaired social interaction and communication and is generally associated with repetitive behavior. About 1% children are estimated to be affected by autism.


Department Cell Biology, Emory University, Atlanta, USA.
*Institute for Stem Cell Biology and Regenerative Medicine (InStem), National Center for Biological Sciences (NCBS), Bangalore 560065, India.
ravism@instem.res.in
Stem cells are cells found in all cell types. In cell biology terms, regulatory information, which cells that can differentiate and cycle in which condensed and align in the middle of the cell carries the message about the three germ layers, endoderm, mesoderm or ectoderm.

Pluripotent stem cells are the cells, which have the potential to differentiate into any of the three germ layers, endoderm, mesoderm or ectoderm.

In FXS, the resultant hypermethylation leads to the transcriptional silencing of the FMR1 gene and loss the protein product of this gene, FMRP. There are two stages in the repeat expansion, premutation (50–200 repeats) and full mutation (>200 repeats). The two phase expansion of the repeats explained the unusual pattern of fragile X inheritance which was termed the Sherman paradox. The premutation (50–200 repeats) also leads to two distinct clinical conditions from FXS. In carriers of premutation, the higher the CGG repeats, the greater the Fmr1 mRNA level. In 40% of older male carriers of such mutation, this causes fragile X associated tremor/ataxia syndrome (FXTAS). In female premutation carriers, high prevalence of ovarian failure is reported, which is referred to as fragile X associated primary ovarian insufficiency (FXPOI). Now it is recognized that the trinucleotide expansion can happen both in coding and non-coding regions of many genes and contribute to several neurological disorders including Huntington disease, myotonic dystrophy and several inherited ataxias.

The cause for the repeat expansion at this particular locus is still unclear but recent findings suggest that even with full mutation, FMR1 allele remains active in the early embryonic stages. In the embryonic stem cells derived from the full mutation samples (hESCs), FMR1 remains unsilenced but in the inducible pluripotent stem cells (iPSCs) generated from the fibroblasts of FXS patient, the gene remains hypermethylated and silenced. These perplexing results indicate that full mutation FMR1 gene remains active in the early embryonic stage but is silenced later on during differentiation. Although iPSCs resemble ESCs on most aspects, few aspects such as hypermethylation of FMR1 gene remain irreversible in iPSC. More recent methods to generate iPSCs representing an earlier development stage, called ground state/naïve pluripotent stem cells, may provide a means to study the mechanism and timing of FMR1 gene silencing resulting from the full mutation. Elucidating the precise time and mechanism of silencing of FMR1 gene with full mutation due to the hypermethylation may provide a clue about the etiology of the disorder and hope to

Figure 1: Influence of FMRP on developmental stages of nervous system.
activate the gene to reverse the phenotype as potential therapeutic approach.

FMR1 is highly conserved across species indicating its critical role in development and function of nervous system. Two best-studied animal models for FXS are generated in mouse and fruit fly (Drosophila). The CGG repeat expansion in mouse Fmr1 gene did not recapitulate the hypermethylation and silencing of the gene as in humans, but targeted deletion of an exon from mouse Fmr1 gene created a knockout (Fmr1 KO) lacking FMRP, and is used extensively as a functional equivalent to model the full human mutation. Similar to FXS patients, these KO mice exhibit disrupted learning and memory, increased susceptibility to seizures and large testes. Recently a conditional KO has been generated by inserting loxP sites flanking exon 1 of Fmr1 gene which enable study of the effect spatiotemporal by regulated knockdown of FMRP. D. melanogastor models are generated by null mutation of fly ortholog dfmr1 gene. These mutant flies have impairment of long-term memory and reduced courtship behavior. The animal models of FXS have been of enormous help in the study of the disorder and in understanding the role of the gene product, FMRP in the normal development and function of nervous system.

2 FXS is a Synaptic Disorder

Fragile X syndrome causes severe cognitive deficiency and developmental delay with a mean average IQ of below 50 in fragile X boys. Surprisingly, this clear disruption in the function was not reflected in postmortem studies of gross anatomical features of the affected brain. There were only very minor changes observed in the brain of the affected individuals compared to control subjects. The prominent neuroanatomical feature observed was the dysgenesis of dendritic spines which look long, thin and likely to be more immature, a feature observed both in the patients and in the mouse model of FXS. Spines are the sites of excitatory synapses, which are short protrusions joined to the main dendritic shaft by a thin neck. Spines are abundant in higher brain regions and highly variable in size and shape. They are reported to be frequently generated and eliminated even in the adult brain and are thought to be substrates for stable memory formation. Relatively unaffected gross anatomical features combined with presence of prominent differences in the dendritic spine structure in the affected individuals suggest defects in the normal synaptic function and plasticity of these connections in FXS. Considering these facts in the context that alteration of dendritic spines represents a common hallmark of mental retardation diseases, FXS can be considered as a synaptic disorder. Thus studying the abnormal spine dynamics and plasticity provides insight into cellular/subcellular basis for FXS.

2.1 Spine phenotype

The abnormal spine phenotype in FXS was first recognized from the brain autopsy of a fragile X patient. The brain autopsy from the patients revealed thin, long and tortuous looking dendritic spines on pyramidal neurons from layers III and V of cortex. The postmortem study from cortical tissue of the patients also revealed increased spines density. Interestingly, the spine phenotype observed in FXS resembles the immature spine precursors, filopodia, suggesting alteration in spine development and function. Spine abnormality observed in patients was recapitulated by the mouse model of FXS, in which thin, long and immature spines were reported from cortex and dentate gyrus of the hippocampus. Increased spine density was also reported from several other brain regions although there is inconsistency about spine density and the brain areas and the age of the animal in which the defect persists. Of special interest are two recent reports which emphasized that the major abnormality in Fmr1 KO (mouse model of FXS) is the augmented spine turnover which fails to decrease in the early postnatal weeks leading to delay in spine stabilization and maturation. The persistence of augmented spine turnover into adulthood in Fmr1 KO may in part explain the abnormal spine phenotype since the transient spines display smaller head and longer necks.

Dendritic spines represent the excitatory synaptic connections between neurons. The spine defects indicate an alteration in synaptic function, strength or development, which may be responsible for the cognitive deficiency in the nervous system observed in FXS. Synaptic dysfunction could result from defects in both pre and post-synaptic terminals. FMRP, a predominantly cytoplasmic protein is actively transported to the dendrites and localized at the post-synaptic termini on the dendritic spines. ENREF_32. The transport and the dynamics of FMRP in dendritic spines are reportedly modulated by neuronal activity.

There is also considerable evidence for the pre-synaptic function of FMRP. The localization of FMRP to growth cones and developing axons was demonstrated to be defective in Fmr1 KO mice where the growth cone filopodia were shown to be more numerous but were less dynamic and motile compared to wild type. In an interesting study
using FMRP and GFP “mosaic” mouse obtained by crossing Fmr1 KO mice with a mice harboring GFP on X chromosome, the investigators recorded synaptically connected pairs of CA3 neurons in organotypic hippocampal slice cultures. They reported that loss of FMRP in the presynaptic neuron led to reduction in the local functional excitatory connections to CA3 neighbors implying a presynaptic role for FMRP. Increased spine density and immature spine phenotype observed in the absence of FMRP along with the reduced axonal projections indicate a possible lack of proper axon pruning based on activity which may explain both pre and postsynaptic deficiencies in the animal models of FXS. Apart from the synaptically defects the excitatory/inhibitory equilibrium in the brain is also affected in the absence of FMRP. Studies on the excitatory and inhibitory circuits in somatosensory cortex of Fmr1 KO mice have reported an overall decrease in excitatory drive in inhibitory neurons at this region. This leads to an increased intrinsic excitability of excitatory neurons resulting in a hyperexcitable circuit in neocortex, which might contribute to many phenotypes observed in FXS.

2.2 Synaptic plasticity defects

Cognitive deficiency, which is a most common feature of FXS, suggests a defect in synaptic plasticity a phenomenon of nervous system thought to be essential for cognition. Synaptic plasticity represents the experience based alterations in number, structure and/or functional efficacy of synaptic connections. The best studied forms of synaptic plasticity are long term potentiation (LTP) where synaptic efficacy is increased based on activity and long term depression (LTD) where synaptic efficacy is decreased upon activity. LTP and LTD are observed in all parts of brain and a host of neurotransmitters and pathways induce and modulate these forms of plasticity. Synaptic plasticity is linked to learning and memory process and is recognized as the underlying cause affected in cognitive deficiencies in many neurological disorders. In animal models of FXS, studies have consistently reported two primary findings: enhanced Gq-coupled receptor dependent LTD and impaired cortical LTP. Among these, enhanced mGluR (metabotropic glutamate receptor)-dependent LTD both in hippocampus and cerebellum of Fmr1 KO mice has attracted maximum attention. mGluR-LTD is mediated by a rapid endocytosis and persistent decrease in surface expression of postsynaptic ionotropic AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid: a subtype of glutamate receptor agonists) receptors, a process which is protein synthesis dependent. If protein synthesis is blocked, mGluRs still trigger the endocytosis of AMPARs, but they are recycled back to surface. The newly synthesized proteins are suggested to maintain decreased surface AMPAR expression by regulating their endocytosis and recycling. In Fmr1 KO mice, the enhanced mGluR-LTD exaggerated and is also protein synthesis independent. Consequently it was suggested that the function of FMRP is to suppress mGluR induced protein synthesis and the absence of FMRP leads to excessive synthesis of these proteins, which will render the mGluR-dependent plasticity, protein synthesis-independent. This led to the proposal of “mGluR theory of fragile X syndrome” where excessive mGluR responses in the absence of FMRP is thought to account for majority of the FXS phenotype. While most functional studies in Fmr1 KO mice are carried out in hippocampus and cortex, the emotional symptoms observed in FXS point to the involvement of amygdala which play a central role in emotional cognition. Recent studies report a reduced AMPAR surface expression in amygdala of Fmr1 KO mice which interestingly leads to impaired mGluR-LTP.

However, mGluR is not the only pathway affected in the absence of FMRP, as activation of Gq-coupled M1 muscarinic acetyl choline receptors (mAchR) induce protein synthesis dependent LTD which is also enhanced in Fmr1 KO mice. GABA (γ- amino butyric acid) and dopaminergic pathways are also impaired in the mouse and fly models of FXS. GABA is a major inhibitory neurotransmitter in the brain and plays an important role in learning and memory. Several subunits of GABA receptors are reduced in the brains of Fmr1 KO mice and in D. melanogaster model of FXS. This may result in the decreased inhibitory input on the excitatory network in the absence of FMRP. Dopamine is an important neurotransmitter in prefrontal cortex. Activation of D1 receptors leads to an increased surface expression of AMPARs which is absent in the neurons from Fmr1 KO mice. Impairment of the dopamine pathway may contribute to the hyperactivity and other motor related phenotypes of FXS.

Initial studies did not detect any hippocampal LTP defects in the absence of FMRP, but more recent work has clearly demonstrated a deficit in the magnitude of LTP both in hippocampus and neocortex of Fmr1 KO mice. Interestingly the LTP studies in Fmr1 KO mice also reported that LTP expression mechanism is intact in these mice but the “threshold” for LTP induction is much higher and might be a cause of the observed deficiency.
Synaptic abnormality has evolved as a central feature of FXS emanating from studies based on animal models. This is manifested in the form of abnormal spine morphology and dynamics, synaptic deficiencies and defective synaptic responses. The exaggerated mGluR response in the absence of FMRP led to the “mGluR theory of FXS” which has been a driving force for the research and targeted therapeutic approach in fragile X research. However, recent reports clearly indicate the necessity to go beyond the mGluR signaling to get a comprehensive understanding of FXS. Exploring the function of FMRP in normal development and function of the nervous system may hold a key to unraveling the etiology of FXS and also for developing and refining the targeted therapeutic design for this disorder.

### 3 Molecular Details of FMRP Function

FMRP is the product of FMR1 gene on the X chromosome localized to the fragile X site originally observed in FXS patients. Trinucleotide repeats expansion in the 5'UTR of FMR1 gene results in the loss of expression of FMRP protein. It is reasonable to conclude that absence of FMRP is the sole cause of the spine abnormality and the defects in the synaptic plasticity observed in FXS. Thus, exploring the function of FMRP also provides an opportunity to study the role of a single gene product, which has such significant impact on the development and plasticity of nervous system. The FMR1 gene comprises 17 exons and is subject to alternate splicing resulting in several isoforms of the protein. The predominant 72 kDa protein is expressed in many tissues including germ cells and neurons. FMRP is highly conserved and has two paralogs (FXR1 and FXR2 proteins) in mammals. While predominantly cytoplasmic, FMRP has conserved nuclear localization (NLS) and nuclear export signals (NES), which predicts a shuttling of conserved nuclear localization (NLS) and nuclear export. While predominantly cytoplasmic, FMRP has paralogs (FXR1 and FXR2 proteins) in mammalian species. FMRP is highly conserved and has two paralogs (FXR1 and FXR2 proteins) in mammals. While predominantly cytoplasmic, FMRP has conserved nuclear localization (NLS) and nuclear export signals (NES), which predicts a shuttling of conserved nuclear localization (NLS) and nuclear export. FMRP is the product of FMR1 gene on the X chromosome localized to the fragile X site originally observed in FXS patients. Trinucleotide repeats expansion in the 5'UTR of FMR1 gene results in the loss of expression of FMRP protein. It is reasonable to conclude that absence of FMRP is the sole cause of the spine abnormality and the defects in the synaptic plasticity observed in FXS. Thus, exploring the function of FMRP also provides an opportunity to study the role of a single gene product, which has such significant impact on the development and plasticity of nervous system. The FMR1 gene comprises 17 exons and is subject to alternate splicing resulting in several isoforms of the protein. The predominant 72 kDa protein is expressed in many tissues including germ cells and neurons. FMRP is highly conserved and has two paralogs (FXR1 and FXR2 proteins) in mammals. While predominantly cytoplasmic, FMRP has conserved nuclear localization (NLS) and nuclear export signals (NES), which predicts a shuttling of conserved nuclear localization (NLS) and nuclear export. While predominantly cytoplasmic, FMRP has paralogs (FXR1 and FXR2 proteins) in mammalian species.

### 3.1 mRNAs associated with FMRP

Characterization of FMRP as an RNA binding protein and its association with polyribosomes led to the hypothesis that in the absence of FMRP, the mRNAs which normally associate with FMRP may be translationally misregulated. Thus began the quest to identify the mRNAs associated with FMRP and relating this association to the phenotype of FXS. In a first major effort, Brown et al performed microarray for the mRNAs immunoprecipitated (IP) from mouse brain using monoclonal antibody specific to C-terminal region of FMRP (mAb 7G1–1). mRNA profile from the Frmr1 KO brain IP was used as a control baseline. Steady state mRNA levels in the input were unchanged between the wild type and Frmr1 KO brains, indicating that mRNA stability may not have been affected by the absence of FMRP. In this assay, 432 mRNAs were identified as possible candidates to associate with FMRP. To correlate the immunoprecipitation data functionally to FMRP, they further compared microarray profiles of mRNAs in the heavy polyribosomes from normal human lymphoblastoid cells and cells derived from fragile X patients. Among the top 80 genes identified in the microarray, 28 genes were expressed in human lymphoblastoid cells, out of which 14 showed a differential distribution in heavy polyribosomes between normal and fragile X cells. While the relatively low number could be due to restricting the assay to heavy polyribosomal fractions and a choice of non-neuronal cells for the assay, this study provided the first reliable list of potential FMRP target mRNAs. In a related study, several of these candidate mRNAs had a distinct G-quartet structure in their 3' untranslated region (UTR), which was shown to interact with RGG box domain of FMRP. The common sequence motif of DWGG-N(0–2)-DWGG-N(0–1)-DWGG-N(0–1)-DWGG was recognized from in vitro RNA selection with histidine tagged-FMRP. Two to four G quartets can stack into a structure, which is stabilized by potassium and sodium but destabilized by lithium. The RGG box is predicted to interact with G-quartet structure present in many mRNAs. The N terminal and central domains of FMRP are also involved in protein-protein interactions. FMRP interacts with many proteins including FXR1/2, Cytoplasmic FMRP Interacting protein (CYFIP) 1 and 2, and Argonaut protein (Ago) 2, 61–63 The extensive protein-protein interactions may explain the presence of FMRP in many multi-protein complexes and RNA granules such as P-bodies and stress granules.

**Polyribosomes:** Polyribosomes are an association of multiple ribosomes on single mRNA molecules. Polyribosomes associated with an mRNA generally imply the actively translating status of an mRNA.

**Microarray:** Microarray is a 2D array on a solid substrate that assays large amounts of biological material using high-throughput screening methods.

**MicroRNAs:** MicroRNAs are recently identified very small non-coding RNA molecules. These are generated from much bigger precursor molecules by a series of processing. MicroRNAs regulate protein synthesis and play critical roles in many aspects during development and function of most multicellular organisms.
quartets can be formed by both intra and intermolecular interactions involving one or more RNAs, although it is thought to be intramolecular in FMRP and mRNA interactions. By identifying sequence/structural specificity for FMRP binding to its potential target mRNAs and providing a functional correlation in the form of misregulated translation of these mRNAs in the absence of FMRP, these studies\textsuperscript{60,67} provided a solid platform for elucidating the molecular mechanism of FXS. Many of the candidates from this list were thought to be involved in neuronal development and synaptic plasticity. However, even a decade after these publications there is only a limited success in establishing a meaningful link between the predicted mRNA targets of FMRP to the phenotype of FXS (Table 1).

Using a candidate-based approach, several laboratories have tried to identify potential mRNA targets of FMRP, using direct and indirect methods, based on their known involvement in synaptic plasticity and spine morphology\textsuperscript{57,68–70} Zalfa et al\textsuperscript{72} showed that $\alpha$-CaMK II, Arc and MAP1B mRNAs interact with FMRP in mouse brain lysate and their translation was dysregulated (as measured by the polysomal incorporation of these mRNAs) in the absence of FMRP. A much contested part of this work was an observation that a small noncoding RNA BC1 or its human analog BC 200 RNAs bind directly to FMRP and this is essential for the interaction of FMRP with selected target mRNAs. Several groups challenged these results\textsuperscript{71,72} and reported that BC1 RNA-FMRP interaction is likely to be nonspecific and the FMRP-target mRNA interactions were unaffected by BC1 RNA. Two groups independently reported\textsuperscript{68,73} that mRNA for postsynaptic density-95 (PSD-95), another important synaptic protein as a target of FMRP by both immunoprecipitation and gel mobility shift assays. However, while Zalfa et al reported stability of PSD-95 mRNA was affected in hippocampus of Fmr1 KO mice,\textsuperscript{73} Muddashetty et al reported a dysregulated translation of PSD-95 in the absence of FMRP.\textsuperscript{68} This reported disparity in the mode of influence of FMRP on PSD-95 mRNA still needs to be effectively reconciled to make a better sense of FMRP function. Focusing on the functional relevance, Muddashetty et al\textsuperscript{68} showed only a subset of dendritic/synaptic mRNAs including PSD-95, GluR1 and 2 (components of AMPA receptors) and CaMKII$\alpha$ are associated with FMRP. The search for mRNAs associated with FMRP based on their functional relevance has led to the identification of several other mRNAs, which are involved in various aspects of synaptic plasticity (Table 1).

Recently in another high throughput analysis of FMRP target mRNAs, Darnell et al. used HITS-CLIP (High Throughput Sequencing Cross Linking IP) assay to search for mRNAs associated with FMRP in vivo.\textsuperscript{74} In this assay, brain slices were cross linked by UV which creates a covalent bond between RNA and protein molecule that are in direct contact. The crosslinking was followed by lysis and separation on sucrose gradient to isolate the polyribosomes. These polyribosomes were further crosslinked and immunoprecipitated with FMRP under stringent denaturing conditions. The crosslinked and immunoprecipitated RNAs were analyzed by high throughput sequencing. 842 transcripts were identified as FMRP targets of which several candidates overlapped with previous microarray based list of FMRP targets.\textsuperscript{60,67} Not surprisingly many of the identified targets code for pre and postsynaptic compartment proteins. They include the components of synaptic signaling pathways, glutamate receptor signaling.

### Table 1: Selected list of FMRP target mRNAs with possible link to FXS phenotype

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Localization</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmr1</td>
<td>Dendritic</td>
<td>Synaptic</td>
<td>(113, 119)</td>
</tr>
<tr>
<td>Map1b</td>
<td>Dendritic</td>
<td>Cytoskeletal rearrangement?</td>
<td>(67)</td>
</tr>
<tr>
<td>Sema3F</td>
<td>Presynaptic</td>
<td>Growth cone guidance</td>
<td>(67)</td>
</tr>
<tr>
<td>Arc</td>
<td>Dendritic</td>
<td>Synaptic plasticity mGluR-LTD</td>
<td>(57)</td>
</tr>
<tr>
<td>CamKII$\alpha$</td>
<td>Dendritic</td>
<td>Synaptic plasticity</td>
<td>(57)</td>
</tr>
<tr>
<td>eIF1A</td>
<td>Dendritic</td>
<td>Synaptic translation</td>
<td>(120, 121)</td>
</tr>
<tr>
<td>GABA$\alpha_0$</td>
<td>Dendritic</td>
<td>Controls excitatory output</td>
<td>(76)</td>
</tr>
<tr>
<td>Rgs5</td>
<td>Dendritic</td>
<td>Regulation of G protein signaling</td>
<td>(76)</td>
</tr>
<tr>
<td>Pdgf</td>
<td>Dendritic</td>
<td>Post synaptic structure and function LTP and LTD</td>
<td>(68, 73)</td>
</tr>
<tr>
<td>GluR1/2</td>
<td>Dendritic</td>
<td>Synaptic plasticity mGluR-LTD</td>
<td>(68)</td>
</tr>
<tr>
<td>Sod1</td>
<td>Cell body</td>
<td>Oxidative stress</td>
<td>(92)</td>
</tr>
<tr>
<td>PIKE, p110b</td>
<td>Dendritic</td>
<td>mGluR signaling</td>
<td>(69, 122)</td>
</tr>
<tr>
<td>NR2A</td>
<td>Dendritic</td>
<td>Spine morphology and synaptic plasticity</td>
<td>(70)</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>Dendritic</td>
<td>Controls excitatory output</td>
<td>(98, 99)</td>
</tr>
<tr>
<td>Nos1</td>
<td>Dendritic</td>
<td>Synaptic plasticity Retrograde message</td>
<td>(93)</td>
</tr>
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neuropathic pain signaling, GABA receptor signaling, CREB signaling, PKA, PLC, RhoA, G protein coupled receptor pathways, suggesting a direct role of FMRP in regulating translation of pre and postsynaptic proteome to affect synaptic plasticity. An important finding from this work was to identify that many of the gene products, which are implicated previously in autism spectrum disorders (such as SHANK3, NRXN1, PTEN and TSC2), were also associated with FMRP. A caveat in this study is that authors do not consider the mRNAs in the non- polyribosomal fractions for the analyses. FMRP is also reported in the lighter fractions on the sucrose gradient and thus by excluding these fractions valuable information is likely to be ignored in this study. Another intriguing aspect of this study was that 66% of mRNA binding by FMRP was within the coding sequence with no particular recognizable specificity. How FMRP interactions with coding sequences allow for selectivity of FMRP to bind a small subset of mRNAs remains a mystery. This finding is in contrast to the earlier finding of G quartet structure on 3'UTR of mRNAs as specificity factor for FMRP binding and also obscures the possible role microRNAs (which generally bind to 3'UTR) play in FMRP mediated translation regulation (Fig. 2).

### 3.2 Role of FMRP in mRNA transport

The role of FMRP in the transport of its target mRNAs remains unclear. Earlier reports did not show any changes in target mRNA localization in Fmr1 KO mice; whereas subsequent studies using sensitive detection techniques have observed altered localization of FMRP target mRNA distribution in the hippocampal layers. A general observation using either quantitative PCR or fluorescent MS2-reporters in recent studies is that there is seldom any alteration in mRNA levels at the steady state either in dendrite or synaptic preparations. In support of this single molecule studies of localized mRNA in Drosophila have revealed that sequences in the UTR of localized RNAs are sufficient to recruit single mRNA to motors. Interestingly however, Fmr-/- mouse neurons were found to be unresponsive to mGuR stimulated transport of MS2 tagged mRNA. Similarly, in Drosphila neurons, mRNA transport in dfmr-/- flies was slow and inefficient and rescue with FMRP led to a dose-dependent increase in motility of mRNA particles. Taken together,
these results seem to indicate a model where the most significant function of FMRP is in regulating and increasing the efficiency of transport in response to stimulus.

Activation of the mGluR pathway increased the transport of FMRP and associated mRNAs, primarily on the microtubule cytoskeleton. Recent evidence supports the notion that FMRP bound mRNAs are translationally repressed during transport on microtubules. Sucrose gradient fractions of microtubule (MT) bound FMRP was associated with mRNPs and not polyribosomes, and these RNP increased upon MT destabilization or translational inhibition. FMRP binding to MTs was RNA dependent suggesting requirement for RNA-dependent conformational changes in FMRP. Since mRNA and miRNA association with FMRP depended on the phosphorylation status of FMRP (see below), phosphorylation due to RNA binding or by inducing conformational change in FMRP by itself may regulate motor and MT binding of FMRP.

3.3 Involvement of microRNAs in FMRP function

The proposed role of FMRP as a translation inhibitor has attracted attention due to the possible connection between FXS and the microRNA pathway. MicroRNAs (miRNA) are a class of small non-coding RNAs which regulate gene expression at post-transcriptional phase by modulating mRNA stability and/or their translation. MicroRNAs are predominantly transcribed by RNA polymerase II and undergo a multistep processing to yield double stranded mature microRNA in the cytoplasm. Argonaut (AGO) family of proteins facilitate the incorporation of one selected strand from this into an RNA-induced silencing complex (RISC) based on their sequence specific imperfect pairing onto their target mRNA. The most common outcome of RISC formation on an mRNA is decreased protein expression, but on rare occasions it has also been reported to cause translational activation. MicroRNA pathway shares many components and concept with siRNA mediated gene silencing by a process generally referred to as RNAi pathway.

Discovery of microRNAs had a dramatic influence on our understanding of post transcriptional gene regulation and it is now speculated that majority of protein synthesis in cells is regulated by microRNAs to varying extent. MicroRNAs are post-transcriptional phase by modulating mRNA stability and/or their translation. MicroRNAs are a class of small non-coding RNAs which regulate gene expression at post-transcriptional phase by modulating mRNA stability and/or their translation. MicroRNAs are predominantly transcribed by RNA polymerase II and undergo a multistep processing to yield double stranded mature microRNA in the cytoplasm. Argonaut (AGO) family of proteins facilitate the incorporation of one selected strand from this into an RNA-induced silencing complex (RISC) based on their sequence specific imperfect pairing onto their target mRNA. The most common outcome of RISC formation on an mRNA is decreased protein expression, but on rare occasions it has also been reported to cause translational activation. MicroRNA pathway shares many components and concept with siRNA mediated gene silencing by a process generally referred to as RNAi pathway. Discovery of microRNAs had a dramatic influence on our understanding of post transcriptional gene regulation and it is now speculated that majority of protein synthesis in cells is regulated by microRNAs to varying extent.

One of the first reports on the link between microRNA and FMRP was from Ishizuka et al in identifying the association of dFMR1 (Drosophila ortholog of FMRP) with the RNAi related apparatus including AGO2 (Argonaut 2) and Dicer proteins in Drosophila. Later Jin et al demonstrated that mammalian FMRP interacts with microRNAs and components of the microRNA pathway including Dicer and AGO2 (also referred as EIF2C2). Furthermore, ~80nt RNA was also co-immunoprecipitated with FMRP which was thought to be the precursor microRNAs raising the possibility that FMRP may also play a role in microRNA processing. The link of FMRP to the microRNA pathway led to the search for specific microRNAs associated with FMRP and their possible contribution to FXS. In Drosophila ovary, bantam microRNA was shown to be associated with dFMR1 and regulate the fate of germ line stem cells. Edbauer et al identified 12 microRNAs associated with FMRP in mouse brain by immunoprecipitation. Only a selected group of 18 microRNAs were sampled in this study based on their expression in the brain and involvement in pathways affecting spine morphology. While the physical link between microRNA pathway and FMRP is quite clear from all these studies, the functional link and its significance for FXS is only beginning to be explored. Among the microRNAs associated with FMRP, miR-125b and miR-132 were shown to have contrasting effect on spine morphology. Overexpression of miR-125b resulted in longer and thinner protrusion while overexpressed miR-132 caused an increase in average protrusion width without affecting the average length. The effect of these microRNAs on spines was abolished if FMRP was acutely knocked down using shRNAs in these cells, indicating that FMRP is required for the microRNA mediated manipulation of spine morphology.

MicroRNAs and FMRP seems to share a common purpose in their function as translation inhibitors and thus it can be proposed that they augment each other’s function bringing about effective translation inhibition. A more interesting hypothesis is that FMRP may subtly alter the function of microRNA pathway to reversibly inhibit the translation of its target mRNAs (see below). In support of this hypothesis, absence of FMRP does not seem to affect the general function of microRNA or RNAi pathways, but it does seem to affect the microRNA mediated regulation of a subset of mRNAs which has important synaptic functions. Mudodashetty et al reported that miR-125a regulates the expression of PSD-95, an important postsynaptic scaffolding protein. miR-125a mediated translation of PSD-95 is in fact regulated by FMRP in neurons. This study also reported, for the first time, the possible role of FMRP in reversing the microRNA mediated translation inhibition in response to specific activation of a neurotransmitter receptor, thus elucidating a
The absence of FMRP has shown to affect the neuron stem cell maintenance and fate determination. Absence of FMRP has been implicated in the regulation of neuron stem cell maintenance and fate determination. In Fmr1 KO mice in comparison to wild type mice, FMRP KO mice showed significantly reduced miR-125a localization to PSD-95 mRNA which may also be the cause of significantly reduced miR-125a localization to dendrites and synapses in Fmr1 KO neurons.

Whether this mechanism is also applicable to other FMRP targets needs to be explored and if so, the shared structural and functional features common among these targets is yet to be determined. It is also not known how microRNAs contribute to the phenotypes of FXS though current studies clearly indicating a role in spine morphology and synaptic plasticity. High throughput analyses of the dendritic/synaptic localization of all the micro-RNAs and their dynamic interaction with RISC in Fmr1 KO mice in comparison to wild type mice could provide valuable information in this regard.

MicroRNAs are known to play a critical role in stem cell maintenance and fate determination. Absence of FMRP has shown to affect the neurogenesis and neuronal differentiation. Process clearly influenced by microRNAs. Thus, a potential functional interplay between FMRP and microRNAs in the early developmental stages of nervous system demands further attention.

Muddashetty et al attributed a significant role to the phosphorylation of FMRP in manipulating microRNA mediated translation of PSD-95 mRNA. The phosphorylated form of FMRP facilitates formation of RISC on PSD-95 mRNA and dephosphorylation of FMRP is required for the dissociation of RISC and reversal of translation inhibition. Cheever and Ceman attributed a different function for FMRP and its phosphorylation in manipulation of microRNA pathway. According to them phospho-FMRP specifically associates with precursor microRNA (80 nucleotides long), thus phosphorylation of FMRP may play role in microRNA processing. They report that phosphorylation of FMRP may block the FMRP-Dicer interaction and P-FMRP bound pre-microRNAs are protected from Dicer mediated processing in to mature microRNAs. This rather intriguing role of FMRP is proposed to provide an explanation for the possible requirement of FMRP for the translation activation in certain cases, which are again based on the rare reports of microRNA involvement in translational activation.

3.4 FMRP and translation

The association of FMRP with polyribosomes and a large number of mRNAs (around 4% total mRNA according to one estimate) indicated a role in the translation. There is considerable data that suggests that FMRP acts as a translational repressor. In one of the first such evidence, FMRP was shown to reduce the translation of various mRNAs in vitro in rabbit reticulocyte lysate and in Xenopus oocytes. In Drosophila, dFMRP represses the translation of futsch, an ortholog of mammalian MAP1B, a well characterized FMRP target mRNA. In mammalian system, several FMRP target mRNAs such as Arc, α-CaMKII, PSD-95 and GluR1/2 were significantly shifted to polyribosomal fractions in synaptosomal preparation from Fmr1 KO mice indicating an enhanced translation compared to wild type. Increased protein synthesis was also reported from Fmr1 KO hippocampal slices. Apart from polyribosomes, FMRP is also reported in stress granules, P bodies and other RNA granules which influence the protein synthesis (ref). It is still not clear whether FMRP has a functional role in these granules or its presence is only a secondary effect. Clearly FMRP posits itself as a significant player to act as a dynamic modulator between different functional domains of protein synthesis and its regulation. Hence deciphering the role of FMRP in neuronal translation is likely to unravel its contribution to synaptic plasticity and thus elucidate the molecular details of FXS phenotype.

While majority of the reports implicate FMRP as a translational repressor, there are few significant reports indicating that FMRP may also act as a translational activator in selected instances. In one such case FMRP was reported to upregulate the translation of superoxide dismutase 1 (Sod1) mRNA. In Sod1 mRNA FMRP is reported to interact with a SoSLIP (Sod1 mRNA stemloop interacting with FMRP) domain on 5′UTR. This domain acts as a mild internal ribosome entry site (IRES) and on its own increases the reporter (luciferase) expression several fold. FMRP is proposed to stabilize SoSLIP structure, but the effect of FMRP on SoSLIP containing reporter expression seems only moderate and the mechanism of translation activation by FMRP is unclear. SOD1 expression is decreased in Fmr1 KO mice and given the important role played by this protein in balancing the oxidative stress in the cells, the contribution of defective SOD1 expression in the absence of FMRP to FXS phenotypes such as anxiety, sleeping difficulty and autism needs further exploration. Potassium channel Kv4.2 is another protein whose expression is positively regulated by FMRP. Gross et al reported significantly reduced levels of Kv4.2 protein in hippocampal neurons of Fmr1 KO mice which was correlated to decreased Kv4.2 mRNA in polyribosomal fractions. Both 3′ and 5′ UTRs of the mRNA were shown to be essential for FMRP mediated positive influence on the translation.
translation of this mRNA, though no specific RNA structure or mechanism was identified to explain this phenomenon. Later Lee et al.99 also reported FMRF association with 3’UTR of Kv4.2 mRNA possibly to a conserved U-rich sequence through its interaction with C-terminal domain. However, unlike the earlier report Lee at al showed an increased translation of Kv4.2 in hippocampal neurons in the absence of FMRF. More recently, Kwan et al.193 reported that FMRF acts as a translational activator of nitric oxide synthase 1 (Nos1) mRNA in developing human neocortex. Nos1 is neuron-specific and its product nitric oxide (NO), is proposed to act as retrograde messenger at the synapse and may play important role in synaptic plasticity.100 Interestingly, FMRF interacts only with the human Nos1 mRNA through the G quartet structure present in the coding region and activates its translation. Severe reduction in Nos1 protein levels was observed in human FXS patients and this deficit was age dependent, being very dramatic in fetal cases, less so in later developmental stages. The mouse Nos1 mRNA does not have this specific FMRF binding site and neocortical Nos1 levels are not affected in Fmr1 KO mice. This report has many striking features with interesting implications. This is the first report on species-specific effect of FMRF and clearly poses a challenge about extrapolating the results from animal models of FXS to human patients specially while designing therapeutic approaches. It is yet to be analyzed whether this species-specific effect is restricted to Nos1 mRNA or there could be other such instances. Previously, the presence of a G quartet structure in the coding region93 was reported to cause FMRF mediated translation inhibition (APP mRNA) but here the same sequence is proposed to act as translation activator. This adds a further complication to the already highly confusing mechanism of FMRF’s influence on the translation of its target mRNAs (see below). Very little is known about the mechanism of FMRF as a translational activator as few reported examples have shed little light on the mechanistic details. Clearly more work is needed to elucidate the role of FMRF as translational activator and the mode of this action. This may help in identifying the subset of its targets, which are translationally activated and their contribution to the FXS phenotype.

There is considerably more information on the mechanism of FMRF as a translation repressor. The evidence that FMRF target mRNAs are shifted to polyribosomal fractions (active elongation complexes) in the absence of FMRF48 indicates that their translation repression is likely to be at pre-initiation step. One possible mechanism is that FMRF blocks the formation of eIF4F complex on its target mRNAs. Formation of eIF4F is an important early step in the translation initiation which involves the interaction between the cap binding protein eIF4E with the scaffolding protein eIF4G, which also interacts with the poly A binding protein (PABP) and thus circularizes the mRNA and promotes ribosome assembly.101 There are many regulatory proteins, which commonly target this step by competing the binding to eIF4E, since this is a limiting factor. Cytoplasmic FMRF interacting protein 1 (CYFIP1) which associates with FMRF is shown to act like an eIF4E binding protein (eIF4E-BP) and inhibits formation of translation initiation complex on FMRF target mRNAs.102 In mice expressing half the normal level of CYFIP1 the expression of proteins such as MAP1B, CaMKIIα and APP (other FMRF targets) were significantly increased further supporting this model. In another model, a small non-coding RNA, BC1 was reported to form a ternary complex with FMRF and its target mRNAs such as MAP1B and CaMKIIα and inhibit their translation at pre-initiation step.57

The association FMRF with microRNAs supports the idea that FMRF target mRNAs are translationally repressed by formation of microRNA induced silencing complex (miRISC). Maddashetty et al.60 reported that in the absence of FMRF, the interaction of miR-125a and its target PSD-95 mRNA with AGO2 (core component of RISC) is significantly reduced and PSD-95 mRNA is translationally de-repressed. According to this report, FMRF in its phosphorylated form, interacts with AGO2 and promotes the formation of miRISC on the 3’UTR of its target mRNA at a pre-initiation step. Mechanism of microRNA mediated translation inhibition is highly debated, but the predominant opinion is that the translation is inhibited at a pre-initiation step.60 Accumulation of miR-FMRP-mRNA complex in the lighter fractions of sucrose gradient at steady state supports the idea that FMRF assisted microRNA mediated translation inhibition occurs at pre-initiation step.63

It is consistently reported that when separated on a sucrose gradient, majority of FMRF is associated with polyribosomes.59,67,74 The defect in FMRF association with ribosomes (or polyribosomes) could impact its function as observed in I304 N mutation. FMRF is reported to be associated with both puromycin sensitive (i.e. translationally active) and puromycin insensitive (stalled) polyribosomes.74,103 If FMRF acts as a translation repressor at pre-initiation step, presence of majority of FMRF in polyribosomal fractions poses a
mechanistic paradox. One explanation is FMRP possibly inhibits translation also at post-initiation step. The presence of FMRP in stalled (puromycin insensitive) polyribosomes supports this, though the mechanism of inhibition is not known. There is no clear explanation for the presence of FMRP in actively translating (puromycin sensitive) polyribosomes. One possibility is the presence of FMRP in stalled or actively translating polysomes depends on its phosphorylation status and they represent functionally different pools where phosphorylated form is stalled and dephosphorylated form is associated with the translating polyribosomes. If phosphorylation of FMRP acts as switch between inhibition and translation activation of its target mRNAs,\textsuperscript{63} then actively translating polysomes with FMRP may represent a transient phase from which they can be switched back to translation inhibition. Another yet unexplored possibility is that actively translating polyribosomes with FMRP may represent a pool where FMRP acts as translation activator instead of repressor (Fig. 3). Finally reports that FMRP inhibits translation at pre and post initiation steps are not necessarily contradictory, but may represent two different phases of regulation, which may be spatio-temporally separated, as discussed below (Fig. 2).

4 FMRP Dynamics and Synaptic Function

FMRP is a multifunctional protein playing roles in the transport, translation and metabolism of its target mRNAs. Its binding to mRNA targets ensures that they are transported, inhibited and translated in response to neuronal activity. An important outcome of FMRP function is to regulate the activity-mediated expression of specific proteins at synapse which in turn influences synaptic plasticity. To bring about this effect, FMRP acts as a very dynamic molecule whose synthesis, transport, phosphorylation and degradation are acutely regulated. Consequently studying the dynamics of FMRP in neurons and particularly at synapse assumes a critical role in understanding the molecular mechanism of FXS (Fig. 3).

4.1 FMRP transport, phosphorylation and degradation

FMRP is primarily phosphorylated on Serine 499(Serine 500 in hFMRP) which leads to

**Figure 3:** FMRP dynamics in neurons. Initial phosphorylation of FMRP may take place in the cell body though the identity of the kinase remains unclear. Phosphorylated FMRP binds to its target mRNAs (and microRNAs?) and is transported to dendrites by microtubule based anterograde motors, transport in to the post synaptic compartment is further aided by actin based motors. At the synaptic compartment activation of mGluR pathway leads to dephosphorylation of FMRP by PP2A which promotes translation of FMRP target mRNAs. Dephosphorylation also promotes the ubiquitination mediated degradation of FMRP. Fmr1 mRNA is localized to dendrites and synaptic compartment and mGluR activation induces FMRP synthesis at synapse. FMRP could be phosphorylated by S6 kinase at synapse in a mGluR dependant process. FMRP is also associated with retrograde microtubule based motors though the functional significance of this is not elucidated. It is hypothesized that FMRP can shuttle between synapse and nucleus (it has both nucleus localization and export signals) in activity dependent manner.
hierarchical phosphorylation of adjacent serines and threonines in the acidic phosphopeptide stretch. Immunoﬂuorescence studies indicate that majority of the granular FMRP in dendrites is phosphorylated. The S499 residue is a molecular switch that upon mGluR activation can be rapidly (in a minute) dephosphorylated by the phophatase PP2a and release the translational inhibition either due to miRNA release or both. Additionally, within minutes of activation of mGluRs a ribosomal S6 kinase dependent increase in phosphorylation of FMRP could be observed. While the PP2a dependent dephosphorylation has also been observed in distal dendrites, we do not know if the rephosphorylation or denovo phosphorylation due to S6 kinase occurs in dendrites or synapses. The acidic phosphopeptide stretch has multiple phosphorylation sites, which may be phosphorylated in a hierarchical manner. The consensus sequence suggests a role for Casein Kinase II and Glycogen Synthase Kinase β (GSK 3β). Drosophila analog dFMR has been shown to be a target of Casein kinase II in vitro and in vivo and human FMRP can be phosphorylated at S500 by casein kinase II, but we still have no clear idea of precise location and mode of FMRP phosphorylation.

FMRP is transported in neurons by multiple microtubule dependent motors. Kinesins KIF3 and the KIF5 have been both identified as motors that transport FMRP in mammals while the dfmr is transported by both kinesins and dyneins. The hypothesis is that FMRP acts as an adaptor to transport mRNAs on these motors. In drosophila, however, dfmr transport on dynein and kinesin required bicaudal, a recruiting protein to the dynein/dynactin complex. Bicaudal increased the efficiency of dfmr transport by increasing run length. However, the function of bicaudal in dendritic arborization of ddc dorsal neurons required the action of FMRP.

Other than the transport towards distal dendrites and derepression of translation, mGluR activation also leads to a robust novel synthesis of FMRP at the synapse. Whether this mGluR activated synaptically synthesized FMRP is immediately rephosphorylated and binds RNA at the synapse or travels to the nucleus via a retrograde motor to ferry more mRNAs is an intriguing question which still remains to be answered. We do know that a minor share of FMRP (4%) was found in the nucleus and nuclear pores suggesting a role in transport in RNA from nucleus. A possible scenario of the whole ‘FMRP transport cycle’ might actually begin by its synthesis at the synapse, nuclear localization, binding and exporting RNA, transporting RNA to synapse in response to activity, dephosphorylation and relief from translational inhibition either due to release from RNA-miRNA or degradation or both.

4.2 FMRP a modulator of synaptic translation

As discussed in earlier sections, there is considerable evidence about FMRP acting as negative influence on the translation of its target mRNAs. Most of this evidence comes from the observation of increased expression of these targets in the absence of FMRP. However, it might be misleading to label FMRP as “translational repressor” since the activity-mediated translation of many of these mRNAs is clearly lost in the absence of FMRP. Thus “translation modulator” may be a better term for FMRP, which facilitates the activity mediated translation by inhibiting the translation at basal state. An important quest is to elucidate how loss of this modulatory function of FMRP contributes to the symptoms of FXS. Majority of FMRP target/associated mRNAs code for synaptic proteins having important role in spine morphology and synaptic plasticity (Table 1). In the past decade the concept of decentralization of protein synthesis in neurons is evolving rapidly as explanation for disease processes. Synaptic proteins can be synthesized locally in response to appropriate signals and influence the structure and function of synapses. Muddashetty et al, using synaptosomal preparation reported that absence of FMRP precludes the mGluR-induced translation of key synaptic components such as α-CaMKII, PSD-95 and GluR1/2. In neurons where translation is decentralized and is spatio-temporally regulated based on the stimulus received, FMRP seems to act as modulator of activity mediated translation. This process involves two steps, localization of required mRNAs at dendritic/synaptic site in a translationally dormant state and activation of the translation in response to an appropriate signal. FMRP is demonstrated to be essential for both these steps for the translation of its targets as elucidated in case of PSD-95 mRNA. FMRP and miR-125a are required to form an inhibitory complex on PSD-95 mRNA which may represent the translationally dormant mRNA complex at synapse. Using phosphomutants, Muddashetty et al demonstrated that phospho-FMRP favors the formation of inhibitory RISC on PSD-95; moreover overexpression of p-FMRP leads to a shift of PSD-95 mRNA into lighter fractions (mRNP) on a sucrose gradient and inhibits the translation. Stimulation of mGluR pathway in neurons or

Phosphomutants: Phosphomutants are generated by mutating the amino acid, which is phosphorylated (serine and tyrosine are the most commonly phosphorylated amino acids). If the site of phosphorylation is Serine, replacing it with Aspartate will generate a constitutively phosphorylated state while replacing with Alanine will generate dephosphorylated state. Phosphomutants are very useful tools to study the effect phosphorylation in the function of a protein.
synaptosomal preparation reverses the microRNA mediated inhibition of PSD-95 mRNA and leads to its translation. This de-repression is executed by FMRP by switching its phosphorylation state. mGluR mediated FMRP dephosphorylation leads to dissociation of AGO2 (and the associated microRNA) from PSD-95 mRNA which is subsequently shifted to polyribosomes and translated. mGluR mediated dissociation of AGO2 and subsequent translation of FMRP target mRNA was also demonstrated through luciferase reporter constructs having PSD-95 3’UTR. Interestingly, FMRP still remains associated with PSD-95 mRNA (though it is unclear whether it is the same FMRP or newly synthesized one) hinting a possibility that rephosphorylation of FMRP, a slow phase response of mGluR activation105,107 may reverse the process by recruiting AGO2 and microRNA to inhibit the translation. Though it is yet to be demonstrated as a common pathway for FMRP target mRNAs, this provides a first mechanical evidence for the function of FMRP as a modulator of synaptic protein synthesis.

Interaction of FMRP with CYFIP1 is also reported to be modulated in a similar manner.102 CYFIP1, which acts as a elf4E binding protein, is recruited on to the mRNAs by FMRP and inhibits its translation at basal state and stimulation with brain derived neurotropic factor (BDNF) or 3,5—dihydroxyphenylglycine (DHPG—an analog of gp I mGluRs) reduces the amount of elf4E associated with FMRP/CYFIP1 complex. The association of FMRP with various RNA granules such as p-bodies, stress granules and polyribosomes64 opens a possibility that mRNAs might shuttle between different RNA granules in response to stimulation. FMRP may regulate this dynamic movement of RNA. Each of these granules may represent different translational statuses of mRNA, and dynamic changes in this status might be a means of translation regulation at synapse. Very little is known so far about the functional consequence of mRNA shuttling between these granules and the involvement of FMRP in this phenomenon and thus is clearly an area demanding further attention.

Since synaptic plasticity involves structural and functional modulation of both pre and post synaptic terminals, it is interesting to note that many of FMRP target mRNAs code for pre-synaptic proteins according to recent HITS-CLIP assay.74 It is unknown whether the expression of these proteins is activity regulated and occurs at axonal terminals. There is evidence for protein synthesis at axon terminals and also presence of FMRP in growth cones; hence FMRP may play a critical role in neuronal growth and differentiation by regulating the translation at growth cones.36,116 Little is known about the presence of FMRP at presynaptic terminals of mature neurons and its impact on translation in these compartments. A recent report shows that FMRP regulates the NOS1 expression in developing neocortex.93 Nitric oxide, the gaseous product of NOS1 activity is known to act as retrograde messenger across post/pre synaptic terminals. Hence it is possible to speculate that FMRP in the postsynaptic terminal could potentially modulate the translation in the presynaptic terminal through retrograde messengers such as nitric oxide. The functional significance of NOS1 expression regulation by FMRP and its possible involvement of regulating pre-synaptic protein expression need to be further explored (Fig. 2).

5 Conclusion and Future Direction

The progress made in scientific exploration of FXS has greatly advanced the targeted therapeutic approaches for the disorder. Consistent support for “mGluR theory” for FXS from various quarters has attracted group I mGluR receptors as important therapeutic target. mGluR5 antagonists are under various stages of clinical trial,4 apart from that downstream signaling cascade components of mGluR pathway such as PI3 Kinase, mTOR, and glycogen synthase kinase 3β (GSK3β) are explored as therapeutic targets.117,118 Another approach is to focus on the GABA and dopaminergic pathways which are likely to contribute to several phenotypes of FXS and a treatment targeted to these pathways may significantly improve the quality of life for FXS patients.4,118 Fragile X research is clearly leading the way of how basic research on the biology of disease helps design meaningful and targeted therapeutic approaches for a neurological disorder which should further boost the studies on other neurodevelopmental disorders including autism.

Contradictions and disagreements are abound in the field of fragile X research which may reflect the huge interest this topic has generated among the scientific community. However, some clarity is essential on certain aspects such as the mode of FMRP interaction with its target RNAs and mechanism of FMRP mediated translation regulation. The areas that are relatively unexplored include the role microRNAs in the FMRP pathways and the role of FMRP in neuronal stem cells and neurogenesis. Further investigation on the role of amygdala and other brain regions is needed for a better perspective about the different brain functions affected in FXS. Further research in these areas may also provide potential therapeutic tools for fragile X syndrome. The observation that...
FMRF acts as a translation activator for selected mRNAs has not been reconciled with its reported negative influence on majority of targets. Appreciating the role of FMRF as an activity mediated translation modulator rather than a repressor may help to comprehend the multi-faceted function of FMRF.

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Ravi S. Muddashetty is a senior fellow at Institute for Stem cell biology and Regenerative Medicine (InStem)/NCBS, Bangalore. Dr. Muddashetty received his PhD at Westfaelische Wilhelms University of Muenster, Germany, for his work on “Identification of protein partners of BCIRNP and their functional significance”. His post-doctoral work in Prof. Gary Bassell’s lab at Emory University, Atlanta, USA was focused on the elucidation of the function of FMRP as a synaptic translation modulator. In a collaborative work with Prof. Stephen warren’s lab, he also studied the role of microRNAs in the etiology of fragile X syndrome (FXS). During his post-doctoral work he received research grants from FRAXA research foundation and National fragile X Foundation. He also received “Rising star” and “Young investigator” awards for his work on fragile X syndrome. In September 2012 he moved to InStem as faculty member where his primary research focus is on “Mechanism of activity mediated protein synthesis and its implication for the development and disorders of nervous system”.

Gary J. Bassell is a Professor in the Departments of Cell Biology and Neurology at Emory University. Before moving to Emory in 2005, Dr. Bassell was a member of the faculty at the Albert Einstein College of Medicine for ten years. Gary received his Ph.D. degree in Cell Biology from the University of Massachusetts Medical School working in the laboratory of Dr. Robert Singer. His postdoctoral fellowship was in Dr. Kenneth Kosik’s lab at the Center for Neurological Diseases of Brigham and Women’s Hospital at Harvard Medical School. Dr. Bassell is a recipient of the Basil O’Connor Scholar Award from the March of Dimes Birth Defects Foundation, the Irma Hirschl Career Scientist Award, the Dana Foundation Award in Brain Imaging, Autism Speaks Trailblazer Award and a NARSAD Distinguished Investigator Award. Dr. Bassell’s laboratory has been studying the mechanism, regulation and function of mRNA transport and local protein synthesis in neurons, with a special interest in spinal muscular atrophy and fragile x syndrome.

Vijayalaxmi C. Nalavadi is a research associate at the Emory University, Atlanta USA, in the lab of Prof. Gary Bassell. She obtained her masters in biochemistry at Karnataka University, Dharwad and completed her PhD at the Indian Institute of Science in the department of Molecular Reproduction, Development and Genetics under the guidance of Prof. Rajan Dighe. She has worked as a post-doctoral fellow in the Westfaelische Wilhelms University of Muenster, Germany, from 2002–2005 in the lab of Prof. Martin Bahler on kinetic analysis of molecular motor, myosin IXb. In Gary Bassell’s lab her research focused on molecular motor based transport of RNA binding proteins and their signaling dynamics. She has received post-doctoral fellowship from the Fragile X Foundation for her post-doctoral research and has 10 publications. Her future research interest is to study the activity based transport of RNA and RNA binding proteins by molecular motors in neurons and other cells.