Published in final edited form as:

Neuroscientist. 2009 October ; 15(5): 549–567. doi:10.1177/1073858409333075.

The State of Synapses in Fragile X Syndrome

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Abstract

Fragile X Syndrome is the most common inherited form of mental retardation and a leading genetic cause of autism. There is increasing evidence in both FXS and other forms of autism that alterations in synapse number, structure and function are associated and contribute to these prevalent diseases. FXS is caused by loss of function of the *Fmr1* gene which encodes the RNA binding protein, FMRP. Therefore, FXS is a tractable model to understand synaptic dysfunction in cognitive disorders. FMRP is present at synapses where it associates with mRNA and polyribosomes. Accumulating evidence finds roles for FMRP in synapse development, elimination and plasticity. Here, we review the synaptic changes observed in FXS and try to relate these changes to what is known about the molecular function of FMRP. Recent advances in the understanding of the molecular and synaptic function of FMRP, as well as the consequences of its loss, have led to the development of novel therapeutic strategies for FXS and related diseases such as autism.

Fragile X Syndrome

In the United States, the prevalence of mental retardation and autism has been estimated at approximately 0.78% of the population (Larson et al., 2001). Although the social impact of these complex diseases is immeasurable, the lifetime economic cost for all mentally retarded individuals born in the U.S. in the year 2000 has been calculated at over \$50 billion (Honeycutt et al., 2004) underscoring the importance for scientific research on the root causes of mental retardation and autism.

The most common form of inherited mental retardation is Fragile X Syndrome (FXS), and affects approximately 1:4000 males and 1:8000 females (O'Donnell and Warren, 2002). Though the severity and manifestation of symptoms of the disease varies, FXS has several common phenotypes: in addition to a reduction in intellectual ability or IQ, which ranges from mild to severe, many FXS patients also display hyperactivity, hypersensitivity to sensory stimuli, anxiety, impaired visuo-spatial processing, and developmental delay. Thirty percent of children with FXS are diagnosed with autism and 2–5% of autistic children have FXS (Kau et al., 2004; Hagerman et al., 2005). In recent years, a number of human genes have been linked to autism, with *FMR1* being one of the most commonly linked (Kaufmann et al., 2004; Hagerman et al., 2005). Furthermore, roughly 25% of FXS patients suffer from epilepsy (Kaufmann et al., 2004; Hagerman et al., 2005), making FXS a leading genetic model of several complex diseases.

Loss of function of the Fragile X Mental Retardation Gene (*FMR1*) causes Fragile X Syndrome

The molecular cause of FXS arises from loss-of-function mutations in the X-chromosome gene, *FMR1* (reviewed in (O'Donnell and Warren, 2002; Garber et al., 2006)). In nearly all cases, the observed mutation is an expansion of a CGG trinucleotide repeat in the promoter region of the gene. In unaffected individuals, the CGG region is repeated 5 to 50 times. Individuals harboring between 50–200 CGG repeats are defined as premutation carriers. The full mutation state is defined as greater than 200 CGG repeats. At this size, hypermethylation of the repeat region leads to the transcriptional silencing of the *FMR1* gene, and loss of the protein product of *FMR1*, Fragile X Mental Retardation protein (FMRP), an RNA binding protein.

Furthermore, intragenic loss-of-function point mutations or deletions also lead to Fragile X Syndrome (De Boulle et al., 1993; Lugenbeel et al., 1995). Thus, it is widely accepted that FXS results from the loss or significant reduction of FMRP function. In support of this view, the phenotypic variation in IQ and physical features of FXS patients is strongly correlated with levels of FMRP in the blood (Tassone et al., 1999; Loesch et al., 2002; Loesch et al., 2003; Loesch et al., 2004). A mouse model of the disease was created by inserting a neomycin cassette into exon 5 of the mouse *Fmr1* gene (Bakker, 1994). Although, it is difficult to model human mental retardation in mice, the *Fmr1* knockout (KO) mouse recapitulates many aspects of the human FXS condition, including hyperactivity, macroorchidism, anxiety and deficits in learning and memory (Bakker, 1994; Paradee et al., 1999; Spencer et al., 2005; Brennan et al., 2006). The *Fmr1* KO mouse model has been instrumental in studies designed to understand the neurobiological underpinnings of FXS, as well as test new treatment strategies for the condition.

Current models regarding the neurobiological changes which underlie FXS are focused on the synapse. This is based in part on the structural synaptic changes which are observed in both human patients and *Fmr1* KO mouse, as well as alterations in synaptic function observed in the mouse model. Furthermore, recent elucidation of the molecular and cellular functions of FMRP has led researchers to the synapse. Therefore, this review will focus on describing the alterations in synaptic structure, function and plasticity of synapses in FXS and we propose potential cellular mechanisms for these changes based on the known functions of FMRP. There have been several excellent recent reviews on the genetics and clinical features of Fragile X Syndrome or molecular functions of FMRP (Bear, 2005; Garber et al., 2006; Penagarikano et al., 2007; Bassell and Warren, 2008).

Fragile X Mental Retardation Protein: A regulator of dendritic protein synthesis?

To understand the etiology of the synaptic phenotypes which accompany FXS, it is first important to discuss the purported function of FMRP. FMRP is an RNA binding protein whose primary function is thought to be regulation of mRNA translation and perhaps transport of mRNA into dendrites (reviewed by (Feng, 2002; Garber et al., 2006; Bassell and Warren, 2008)). FMRP is found in nearly all cell types of the body, and is expressed particularly strongly in neurons, with minimal expression in glia (Feng et al., 1997a). Although FMRP has functional nuclear localization and export elements, FMRP is primarily found in the cytoplasm (95%) where it is localized to the soma, dendrites, and post-synapse (Eberhart et al., 1996; Feng et al., 1997a; Bakker et al., 2000; Antar et al., 2004). FMRP has also been observed in both axonal growth cones and some mature axons (Antar et al., 2006; Price et al., 2006). Consequently, there is evidence that FMRP may regulate growth cone guidance and/or presynaptic function (Pan et al., 2004; Antar et al., 2006; Hanson and Madison, 2007).

FMRP is an RNA binding protein

FMRP interacts with RNA through several RNA-binding motifs, two hnRNP-K homology domains (KH domains, KH1 and KH2), an arginine/glycine-rich RNA-binding motif (RGG box) and a recently discovered N-terminal domain of FMRP (NDF) (Ashley et al., 1993; Gibson et al., 1993; Siomi et al., 1993; Adinolfi et al., 2003; Zalfa et al., 2005). The KH2 domain is particularly interesting because a single site mutation in the KH2 domain (an isoleucine to asparagine substitution at residue 304; I304N) in FMRP results in a severe form of Fragile X Syndrome in one patient (De Boulle et al., 1993). In addition to disrupting interaction with “kissing complex” RNA structures, I304N FMRP no longer associates with polyribosomes or regulates translation (Feng et al., 1997b; Lagerbauer et al., 2001; Darnell et al., 2005b). These results suggest that disruption of FMRP-RNA interactions or regulation leads to Fragile X Syndrome. FMRP is thought to bind to as many as 400–600 different brain mRNAs, including its own message (Brown et al., 2001; O'Donnell and Warren, 2002) and associates with large, translating polyribosome complexes in brain in an RNA-dependent manner (Khandjian et al., 1996; Tamanini et al., 1996; Feng et al., 1997b; Feng et al., 1997a; Khandjian et al., 2004; Stefani et al., 2004). FMRP is associated with polyribosomes throughout neurons, including dendrites and spines and may be particularly important for translational regulation of dendritic mRNAs (Feng et al., 1997a; Antar et al., 2004). FMRP is also present in smaller mRNA ribonucleoprotein complexes (mRNP) and dendritic “RNA granules,” which are thought to be translationally arrested complexes of ribosomes, RNA-binding proteins, and RNAs, which travel to dendrites on microtubules (Kanai et al., 2004; Antar et al., 2005). Interestingly, FMRP may shuttle between the mRNP and polyribosomes depending on the translational state of the cell (Vasudevan and Steitz, 2007; Wang et al., 2007). It is therefore thought that FMRP may play a role in the local regulation of protein expression at individual synapses remote from the cell body (Fig. 1) (Bassell and Warren, 2008; Ronesi and Huber, 2008a).

Due to the fact that FMRP is an RNA binding protein, a key to understanding how FMRP regulates the nervous system and the effects of FMRP loss is the identity of the relevant mRNA targets. Many studies have identified interacting mRNAs of FMRP using different methods (Sung et al., 2000; Brown et al., 2001; Darnell et al., 2001; Dolzhanskaya et al., 2003; Miyashiro et al., 2003; Zalfa et al., 2003). However, only a handful of mRNAs have been validated as *in vivo* FMRP targets or shown to be misregulated in *Fmr1* KO mice (reviewed in (Darnell et al., 2005a; Bassell and Warren, 2008)). Of particular interest are FMRP target mRNAs which are present in the dendrite and may play a role in the postsynaptic regulation by FMRP. Some of these include the *Fmr1* mRNA itself, microtubule associated protein 1b (MAP1b), postsynaptic density protein 95kDa (PSD-95), activity-regulated cytoskeletal protein (Arc), amyloid precursor protein (APP), elongation factor 1a (EF1a), AMPA Receptor subunits GluR1 and 2, and α Ca²⁺/Calmodulin-dependent kinase II (Brown et al., 2001; Darnell et al., 2001; Sung et al., 2003; Todd et al., 2003; Lu et al., 2004; Hou et al., 2006; Muddashetty et al., 2007; Zalfa et al., 2007; Bassell and Warren, 2008; Liao et al., 2008). Although yet to be validated, numerous additional mRNAs for synaptic proteins, both pre- and postsynaptic, have been identified as putative mRNA targets, suggesting that FMRP may regulate synaptic structure and function through processing, localization or translational regulation of mRNAs encoding pre- and postsynaptic proteins (Brown et al., 2001; Darnell et al., 2001; Miyashiro et al., 2003).

Recent works finds a role for FMRP in the dendritic transport of its mRNA targets (reviewed in (Bassell and Warren, 2008) (Fig. 1). FMRP and associated mRNAs are co-transported into dendrites under basal conditions and in response to neuronal activity (Antar et al., 2004; Antar et al., 2005). Without FMRP, steady state levels of dendritic mRNAs are normal, but RNA granules are less motile and there is a deficit in activity-dependent dendritic mRNA transport

in cultured *Fmr1* KO hippocampal neurons or the Fragile X fly model (Dictenberg et al., 2008; Estes et al., 2008). Dictenberg et al., (2008) identified a C-terminal portion of FMRP that interacts with kinesin light chain (KLC) and expression of this FMRP C-term competes with endogenous FMRP for binding to KLC and blocks transport of FMRP target mRNAs into dendrites (Davidovic et al., 2007; Dictenberg et al., 2008). FMRP association with its targets may also function to stabilize some mRNA targets, such as that for PSD-95 (Zalfa et al., 2007).

Exactly how FMRP regulates translation of its mRNA targets is not entirely understood, but there is evidence that FMRP may act as both a translational suppressor and activator. FMRP-mediated translational suppression of mRNAs in granules is likely important to avoid inappropriate expression of proteins during transport into dendrites (Kindler et al., 2005; Wells, 2006). Evidence for FMRP as a translational suppressor comes from both *in vitro* translation assays and *in vivo* experiments in *Fmr1* KO mice (Laggerbauer et al., 2001; Li et al., 2001; Qin et al., 2005a). The brains of *Fmr1* KO mice exhibit increased protein synthesis rates and an increased association of dendritic mRNAs, such as PSD-95, Arc and GluR1 with translating polyribosomes in comparison to wild-type mouse brains (Qin et al., 2005a; Hou et al., 2006; Muddashetty et al., 2007; Zalfa et al., 2007). One proposed mechanism for FMRP-mediated translational suppression is through association with short noncoding RNAs or microRNAs (miRNAs) which suppress translation by base-pairing with partially complementary mRNA sequences and interaction with proteins in the RNA-induced silencing complex (RISC). The Argonaute proteins, which are a part of the RISC, associate with FMRP, further supporting that FMRP suppresses translation through miRNAs and a RISC-dependent mechanism (Caudy et al., 2002; Jin et al., 2004). Identity of the specific miRNAs that interact with FMRP is unknown. Recent work provides mechanistic insight into how FMRP suppresses the translation machinery. FMRP interacts with Cytoplasmic FMRP-interacting protein (CYFIP1), which in turn binds to the 5' cap binding protein eIF4E, and prevents formation of the eIF4F initiation complex (Schenck et al., 2001; Napoli et al., 2008).

Once mRNAs reach their dendritic destination, FMRP may also facilitate their translation in response to synaptic activity. In support of this hypothesis, a fraction of FMRP is associated with translating polyribosomes in brain and protein synthesis in response to activation of group 1 metabotropic glutamate receptors (mGluRs) is absent in the *Fmr1* KO mice (Feng et al., 1997a; Todd et al., 2003; Khandjian et al., 2004; Stefani et al., 2004; Weiler et al., 2004; Hou et al., 2006; Ronesi and Huber, 2008b) (Fig. 1). Consequently, mGluR dependent plasticity of synaptic and neuronal function is altered in *Fmr1* KO mice (Huber et al., 2002; Koekkoek et al., 2005; Hou et al., 2006). The role of FMRP in mGluR stimulated protein synthesis and plasticity will be discussed in the context of synaptic plasticity below.

Fragile X Syndrome: A defect in synapse elimination?

Human studies

Some of the first neuroanatomical findings associated with mental retardation were alterations in dendritic spine structure (Marin-Padilla, 1972; Purpura, 1974). Dendritic spines are the point of excitatory postsynaptic contact suggesting alterations in synaptic function, strength or development underlie the cognitive dysfunction. Alterations in dendritic spine number, shape and size are common among cognitive disorders, including FXS, Rett's and Down Syndromes (Kaufmann and Moser, 2000).

The first such evidence of altered synapse structure in FXS came from analysis of post-mortem cortical tissue, which revealed an increased number of dendritic spines relative to control individuals (Rudelli et al., 1985; Hinton et al., 1991; Wisniewski et al., 1991; Irwin et al., 2001). These data suggested that excitatory synapse number was increased in FXS patients and

further provided a potential mechanism for the increased rates of epilepsy in FXS. It was additionally noted that a large proportion of the spines of FXS patients appeared abnormally long, thin, and tortuous, a phenotype reminiscent of the immature spine precursors, filopodia, and indicative of alterations in synapse development and/or function (Fiala et al., 1998). It is still not clear if the excess filopodia-like spines in FXS represent functional synapses or immature synapse precursors.

Fragile X Syndrome mouse model studies

Work in the *Fmr1* KO mouse has largely confirmed the spine phenotype observed in FXS patients (Nimchinsky et al., 2001; Irwin et al., 2002; Galvez and Greenough, 2005; McKinney et al., 2005). However, the existence and/or magnitude of the spine alterations in the *Fmr1* KO mouse varies according to brain region, developmental age and genetic background indicating the complex and multi-factorial regulation of spines. Several studies agree that there is an increase in spine number in mature neocortical neurons, but this appears to be sensitive to genetic background (Irwin et al., 2002). The C57Bl6 mouse strain of *Fmr1* KO best recapitulates the human spine differences because adult neocortical neurons (both layer 2/3 and layer 5) display increased spine number, as well as spine length (Galvez and Greenough, 2005; McKinney et al., 2005; Dolen et al., 2007; Hayashi et al., 2007) (Fig. 3). There also appears to be a developmental regulation of the spine phenotype. In very young neurons (1 week postnatal) there is an increased spine density, as well as longer spines in somatosensory layer 5 pyramidal neurons of the FVB strain of *Fmr1* KO mice in comparison to wildtype mice. The dendritic spine alterations are absent in adolescent mice, but reappear in the adult (Nimchinsky et al., 2001; Galvez and Greenough, 2005). Indeed other studies have reported the elongated spines in adolescent or adult neocortex, with no increase in spine density (Restivo et al., 2005; Meredith et al., 2007).

Several studies have observed an increase in the number of long, thin spines and a decreased number of short, stubby spines on neocortical neurons in *Fmr1* KO mice (Irwin et al., 2002; McKinney et al., 2005; Restivo et al., 2005; Meredith et al., 2007). In contrast to neocortex, mature CA1 hippocampal *Fmr1* KO neurons *in vivo* possess more stubby spines and fewer long, thin spines with no change in spine density (Grossman et al., 2006). Stubby spines with large heads are associated with increased synaptic strength (Matsuzaki et al., 2001; Okabe et al., 2001; Kopec et al., 2006), suggesting that CA1 neurons of *Fmr1* KO mice have increased excitatory drive similar to neocortical neurons with excess spine density.

How might the absence of FMRP lead to altered spine number and structure? FMRP may regulate synapse formation, maintenance and/or elimination. Currently it is unclear which process (or processes) FMRP regulates. It has been hypothesized that the absence of FMRP leads to a deficit in synaptic pruning, resulting in an overabundance of synapses (Weiler and Greenough, 1999; Bagni and Greenough, 2005; Antar et al., 2006). Early neocortical synapse development is characterized by an excess production of synaptic connections (Rakic et al., 1986; Markus and Petit, 1987; Grutzendler et al., 2002; Zuo et al., 2005a; Zuo et al., 2005b). During adolescence, an elimination, or pruning, of spines or synapses occurs which requires sensory experience (Zuo et al., 2005b). Additional studies implicate FMRP in larger scale, dendritic pruning. In the mouse somatosensory barrel cortex, immature spiny stellate cells extend their dendrites into both the hollow and septa of the cortical "barrels." During development, septal-oriented protrusions are normally eliminated; however, *Fmr1* KO mice display a failure to prune these septal-oriented dendrites (Galvez et al., 2003). It is important to note, however, that the developmental pruning of multiply-innervated climbing fiber/Purkinje cell connections to singly-innervated connections appears to be normal or even slightly accelerated in *Fmr1* KO mice (Koekkoek et al., 2005). It is therefore possible that FMRP may have a region- or cell type-specific role in postsynaptic pruning mechanisms.

Although the lifelong loss of FMRP in humans and mice results in altered dendritic spines, it was unknown if FMRP directly regulated synapse number, function, or maturation in a cell autonomous fashion. Recent work demonstrated that acute, postsynaptic expression of FMRP negatively regulates synapse number in hippocampal pyramidal neurons (Pfeiffer and Huber, 2007) (Fig. 2). Developing hippocampal *Fmr1* KO neurons in culture display increased synapses, as assessed by immunocytochemical markers. Acute expression of FMRP in *Fmr1* KO neurons resulted in a loss of synapses at both the functional and structural level. Interestingly, measures of functional synapse maturation, such as NMDA receptor properties or the percent of “silent” synapses, were not affected by acute FMRP expression. Furthermore, synapse elimination was not seen following expression of single point-mutant forms of FMRP, such as I304N or one which mimics phosphorylation at the Ser500 residue, S500D (Pfeiffer and Huber, 2007). The latter indicates that postsynaptic FMRP interactions with translating polyribosomes are important for FMRP regulation of synapse number. Expression of the C-terminus of FMRP, which contains the KLC binding domain and blocks dendritic transport of FMRP target mRNAs, results in an increase in the number and length of dendritic filopodia, precursors to synapses, suggestive of a role for dendritic mRNA and translation in this process (Dichtenberg et al., 2008).

Evidence for a pruning function for *Drosophila* FMRP (dFXR)

Much of the evidence for a role for FMRP in synaptic and neurite pruning comes from the *Drosophila melanogaster* model of Fragile X Syndrome. In mammals, FMRP has two autosomal homologs, Fragile X Related Proteins 1 and 2 (FXR1 and FXR2), which share ~60% amino acid identity (Hoogetveen et al., 2002) and likely function similarly to FMRP, in that they have similar RNA binding motifs and associate with FMRP in RNA granules (Bakker et al., 2000; De Diego Otero et al., 2002; Kanai et al., 2004). In *Drosophila*, Fragile X Related Protein (*dFXR*), is thought to serve the function of mammalian FMRP and FXRs (Zarnescu et al., 2005). Consequently, the phenotypes observed in the *dFXR*-mutant *Drosophila* lines, are typically more severe or even different from the mouse and human phenotypes.

In support of a pruning function for dFXR, most neurons of dFXR null flies exhibit an overgrowth and elaboration of axons and dendrites in both the peripheral and central nervous system (Zhang et al., 2001; Gao, 2002; Morales et al., 2002; Lee et al., 2003; Pan et al., 2004; Zhang and Broadie, 2005). The neuromuscular junction (NMJ) has been most well characterized where in addition to increased axon branching and presynaptic bouton number there is enhanced synaptic function consistent with an increase in presynaptic neurotransmitter release and/or synapse number (Zhang et al., 2001; Gatto and Broadie, 2008). Both the pre and postsynaptic effects of the dFXR null are thought to be due to loss of translational suppression of regulators of the cytoskeleton including microtubule associated protein 1B (MAP1B), Rac1 and Profilin (Zhang et al., 2001; Lee et al., 2003; Reeve et al., 2005). This work strengthens a role for dFXR-mediated translational suppression in its neuronal function and highlights dFXR as a regulator of cytoskeletal components. In addition to regulation of synapse structure and number, dFXR regulates the number and type of postsynaptic glutamate receptor subunits at the NMJ. In the dFXR null fly, total glutamate receptor (GluR) levels at the NMJ are unchanged but the relative abundance of the A-class and B-class GluRs subtype are affected in opposing ways (GluRA accumulates and GluRB is lost) (Pan and Broadie, 2007).

Two recent papers characterized the synaptic locus, the developmental and experience-dependent role of dFXR in synaptic development in *Drosophila* (Gatto and Broadie, 2008; Tessier and Broadie, 2008). Constitutive presynaptic expression of dFXR rescued the excessive axon branching and excess presynaptic bouton number, but not increased synaptic function pointing to roles for both pre- and postsynaptic dFXR in development of the NMJ. Early induction of dFXR (during synaptogenesis) completely rescued the axon and bouton

overgrowth (Gatto and Broadie, 2008). In contrast, acute induction of dFXR at the mature NMJ partially rescued increased bouton number, but not branching. These results indicate a role for dFXR in proper axon and bouton development, and a more limited role of dFXR in synapse maintenance (Gatto and Broadie, 2008). The axon pruning role of dFXR in the central nervous system, specifically, mushroom body neurons, as well as dFXR expression, depends on sensory experience (Tessier and Broadie, 2008). This work is the first to provide evidence for role for dFXR in experience and activity-dependent axon pruning. Visual experience drives expression of mammalian *Fmr1* mRNA, suggesting that FMRP may play a similar role in the mammalian visual cortex (Gabel et al., 2004). In support of this idea, deficits in neocortical plasticity in response to sensory deprivation have been reported in the *Fmr1* KO, discussed below (Dolen et al., 2007; Bureau et al., 2008).

Evidence for a presynaptic role of FMRP in formation and/or maintenance of local synaptic connectivity

More recent and functional characterization of the *Fmr1* KO mice has revealed a role for FMRP in establishment and/or maintenance of synaptic connections, perhaps due to a presynaptic function of FMRP. Hanson and Madison (2007) devised a clever strategy to cross the *Fmr1* KO mice with mice harboring GFP on the X chromosome (Hanson and Madison, 2007). The heterozygous female offspring had a “mosaic” co-expression of FMRP and GFP, in essence GFP should act as a reporter for FMRP expression and allowed the investigators to record from synaptically connected pairs of CA3 neurons in organotypic hippocampal slice cultures which varied with regard to their pre- or postsynaptic expression of FMRP. Surprisingly, they reported that the presynaptic loss of FMRP led to a reduction in the local, functional excitatory connections to CA3 neighbors (Hanson and Madison, 2007). In support of these findings, two studies of neocortical synaptic connectivity observed reduced synaptic connectivity in *Fmr1* KO mice (Bureau et al., 2008; Gibson et al., 2008). Using dual whole cell patch recordings from synaptically coupled layer 4 neurons in the somatosensory cortex, a reduced number and strength of functional synaptic connections was observed from spiny stellate excitatory neurons onto both neighboring excitatory and fast-spiking inhibitory neurons in layer 4. Interestingly, the greatest deficit in excitatory drive was onto the inhibitory neurons (~ 50% decrease; Fig. 3) (Gibson et al., 2008). In contrast to the local excitatory connections, the strength of thalamically-evoked excitatory synapses onto L4 inhibitory neurons was normal. Because the excitatory connectivity deficit was common to targets of the L4 spiny stellate neurons, this suggests a role for presynaptic FMRP in L4 spiny stellate axons in the formation or maintenance of synaptic connections with their targets. In support of this idea, Bureau et al., (2008) used laser uncaging of glutamate to map neocortical connectivity and observed decreased connectivity of L4 neuron onto L2/3 pyramidal neurons in the somatosensory cortex. The decreased L4->L2/3 connectivity in *Fmr1* KO resembles the connectivity in a sensory deprived cortex of a wildtype mouse. Whisker deprivation fails to decrease functional connectivity of L4->L2/3 in the *Fmr1* KO mice, perhaps because the *Fmr1* KO cortex is already in a deprived state (Bureau et al., 2008). Interestingly, the L4->L2/3 connectivity deficit was developmentally restricted and was normal by 4 weeks of age. Whereas, the L4->L4 local connectivity deficit persisted for at least 4 weeks, suggesting it may be permanent (Gibson et al., 2008).

How to reconcile the findings of decreased connectivity with the structural “overgrowth” phenotype of the FXS mouse and fly? Bureau et al., observed that the axonal arbors from layer 4 neurons in the *Fmr1* KO neurons were more diffuse and sparse. Specifically, the density of axonal projections was reduced in the center of the barrel and increased along the barrel borders. These diffuse axonal projections are consistent with a lack of proper axon pruning that may result in reduced appropriate synaptic connectivity at the center of the barrel (Fig. 3). A presynaptic role of FMRP in neocortical synaptic connectivity is suggested from these studies,

and is supported by data demonstrating FMRP localization to growth cones and developing axons and FMRP interaction with a mRNAs for presynaptic proteins (Brown et al., 2001; Antar et al., 2006) (Fig. 1). In *Fmr1* KO mice, growth cone filopodia of are more numerous, but are less dynamic and motile in comparison to wild-type mice which may lead to a reduction in synapse formation or maintenance (Antar et al., 2006). The work of Hanson and Madison (2007) and others suggests that FMRP has a presynaptic role in establishment and/or maintenance of local synaptic connectivity. Whereas, the observations of enhanced dendritic spine number in FXS models and loss of synapses with acute postsynaptic expression of FMRP highlight a postsynaptic role for FMRP in synapse elimination (Irwin et al., 2002; Pfeiffer and Huber, 2007). The diverse functions of FMRP in the nervous system is not surprising due to the number and diversity of its interacting mRNAs. Future experiments using acute and cell autonomous manipulation of FMRP in identified neuron populations is necessary to define and reconcile the current, but apparently disparate findings.

Hyperexcitable circuit function in Fragile X Syndrome

Although there are many synaptic phenotypes observed in FXS, the effects of these synaptic changes on overall circuit function is most relevant to understanding how alterations in brain function mediate behaviors observed with this disease. To fully understand circuit function in FXS, it is important to evaluate all components of the circuit, including inhibitory circuit function. Due to the co-morbidity of epilepsy with FXS, as well as the sensory hypersensitivity, it has been hypothesized that there is a circuit hyperexcitability (Miller et al., 1999; Musumeci et al., 1999; Musumeci et al., 2000; Hagerman, 2002).

Most relevant to the sensory hypersensitivity that occurs with FXS are studies examining circuitry in the sensory neocortex. As mentioned above, a large (~50%) deficit in local excitatory drive is observed onto fast spiking interneurons in L4 somatosensory cortex. In contrast, the reciprocal inhibitory postsynaptic currents (IPSCs) are normal (Fig. 3) (Gibson et al., 2008). Because of the profound decrease in excitatory drive onto local inhibitory neurons, it was predicted there would be a decrease in feedback inhibition in the neocortex. This deficit in feedback inhibition together with the findings of increased intrinsic membrane excitability of L4 excitatory spiny stellate neurons would be expected to lead to a hyperexcitable circuit. Consistent with this idea, stimulation of thalamocortical projections into L4, mimicking a sensory stimulus, lead to prolonged neocortical circuit activity, consistent with greater circuit excitability (Gibson et al., 2008). Although no decreases in local inhibitory synapse function were detected, *Fmr1* KO mice are reported to have decreased parvalbumin-positive interneurons, typically fast-spiking, in nearly all layers of somatosensory cortex (Selby et al., 2007). However, decreases in parvalbumin immunoreactivity does not necessarily equate to fewer inhibitory neurons, but could be due to a reduction in parvalbumin protein levels in the neurons (Jiao et al., 2006). Additional evidence for decreased GABAergic function comes from studies demonstrating decreases in specific GABA_A receptor subunits (either mRNA or protein; reviewed in (D'Hulst and Kooy, 2007). Overall, the decrease in excitatory drive onto inhibitory neurons, increased intrinsic excitability of excitatory neurons, and decreases in inhibitory neuron number and increased spine number on L5 neurons would be expected to result in a hyperexcitable circuit in sensory neocortex (Fig. 3). These changes may underlie the sensory hypersensitivity that is prevalent in Fragile X Syndrome. It will be important in future studies to determine if similar hyperexcitable circuit changes are prevalent to other neocortical layers and related brain regions such as the hippocampus. As discussed below, a group 1 metabotropic glutamate receptor triggered epilepsy is dramatically enhanced in hippocampal CA3 neurons of *Fmr1* KO mice, indicating that hyperexcitability is prevalent in the Fragile X brain (Chuang et al., 2005).

Other studies have observed alterations in inhibitory neuron function in both the subiculum and the striatum. Consistent with a hyperexcitability of circuits in Fragile X, Curia et al., (2008) demonstrated a deficit in tonic GABAergic inhibition, but not synaptically released GABA (spontaneous IPSCs) onto subicular pyramidal neurons of *Fmr1* KO mice (Curia et al., 2008). The loss of tonic inhibition suggests a decrease in extrasynaptic GABA_A receptor function. Interestingly, there is evidence for *increases* in GABAergic miniature IPSCs onto medium spiny neurons in the striatum (Centonze et al., 2007). Because these striatal neurons are themselves GABAergic projection neurons, increased inhibitory drive onto these neurons would result in a disinhibition or hyperexcitability of the striatal output.

Changes in Synaptic Plasticity in FXS

The most common mental phenotype associated with FXS is a loss of cognitive ability. A prevailing view in neuroscience is that the phenomenon of synaptic plasticity – the ability of neurons to persistently strengthen (long-term potentiation, LTP) or weaken (long-term depression, LTD) individual synaptic connections in response to activity patterns – is a molecular mechanism underlying memory and cognition. Therefore, many studies have investigated whether the loss of FMRP results in impairments or alterations in synaptic plasticity. Two principle findings have emerged from this work: enhanced Gq-coupled receptor-dependent LTD and impaired cortical LTP in *Fmr1* KO mice.

Gq-dependent LTD

Activation of Gq-coupled neurotransmitter receptors induces a long-term depression of synaptic transmission (LTD) in several regions of the brain (reviewed in (Massey and Bashir, 2007; Bellone et al., 2008)). The most well-characterized of these are the group 1 metabotropic glutamate receptors (mGluRs), mGluR1 and mGluR5, which mediate persistent changes in neurons and synaptic function and therefore are implicated in many forms of brain plasticity, including learning and memory, drug addiction, and chronic pain (reviewed in (Grueter et al., 2007; Dolen and Bear, 2008; Goudet et al., 2008)). Most relevant to FXS, mGluR1 and 5 are canonically linked to translational activation in neurons and stimulate rapid synthesis of FMRP at synapses (Weiler and Greenough, 1993; Kacharmina et al., 2000; Banko et al., 2006; Park et al., 2008). Consequently, a common mechanism by which long-term synaptic plasticity is induced by group 1 mGluRs is through rapid and likely “local” protein synthesis (Merlin et al., 1998; Raymond et al., 2000; Huber et al., 2001; Karachot et al., 2001; Mameli et al., 2007; Waung et al., 2008). Notably, mGluR- induced LTD of CA1 hippocampal synapses requires rapid dendritic synthesis of pre-existing mRNAs (Huber et al., 2000).

Due to the link of mGluRs with FMRP, mGluR-dependent LTD was evaluated in the *Fmr1* KO mice and was found to be enhanced in both the hippocampus and cerebellum (Huber et al., 2002; Koekkoek et al., 2005; Hou et al., 2006). Similarly, activation of the Gq coupled M1 muscarinic acetylcholine receptor (mAChR) induce protein synthesis dependent LTD which, like mGluR-LTD, is also enhanced in *Fmr1* KO mice (Massey et al., 2001; Volk et al., 2007). These findings, combined with evidence for FMRP as a translational suppressor, suggested that FMRP acted to inhibit translation of proteins that were required for Gq-dependent LTD, termed “LTD proteins”. Because one of the proteins synthesized in response to mGluR activation is FMRP itself, it was suggested that FMRP may function as a negative feedback mechanism to limit mGluR-stimulated translation. In addition to mGluR-LTD, mGluR-induced epilepsy, measured as prolonged bursting of CA3 neurons also relies on protein synthesis and is dramatically enhanced in *Fmr1* KO mice (Chuang et al., 2005). From these findings, it was suggested that FMRP may generally function to suppress mGluR and protein synthesis dependent plasticity, termed the “mGluR theory of Fragile X Syndrome” (Bear et al., 2004). Therefore, group 1 mGluR antagonism has been proposed as a

therapy for FXS, for which there is remarkable experimental support (reviewed in (Dolen and Bear, 2008)).

FMRP and Metabotropic Glutamate Receptors

What is known about the role of FMRP in mGluR-stimulated translation and plasticity? As mentioned above, to obtain localized protein expression RNA binding proteins like such as FMRP may serve two functions: 1) to suppress translation of mRNAs during transport and, 2) to function as a modifiable switch to allow or stimulate mRNA translation at the right time and place (Kindler et al., 2005; Wells, 2006). There is some evidence to support a role of FMRP in mGluR-stimulated protein synthesis in this capacity. mGluRs stimulate movement of FMRP and target mRNAs into dendrites where FMRP may function to facilitate transport and/or suppress translation of targets during transport (Antar et al., 2004; Dictenberg et al., 2008). However, at individual synapses or dendritic spines mGluRs stimulate FMRP translation, as well as a rapid ubiquitination and degradation of FMRP, which results in a net decrease in spine FMRP abundance (Weiler and Greenough, 1999; Antar et al., 2004; Hou et al., 2006) (Fig. 1). The purpose of the rapid and bidirectional regulation of FMRP by mGluRs is unclear. Possibly, the loss of synaptic FMRP may function to de-repress mRNA targets and allow translation. In addition to regulation of FMRP abundance, posttranslational modifications of FMRP, such as phosphorylation, or recruitment of FMRP from a translationally inactive mRNP or RNA granule to polysomes may “switch” its function from a suppressor to an activator of protein synthesis (Ceman et al., 2003; Wang et al., 2007). Consistent with this view, recent work demonstrates that mGluRs activate a protein phosphatase, PP2A, and rapid dephosphorylation of FMRP, which in turn stimulates translation of an FMRP target mRNA (Narayanan et al., 2007; Narayanan et al., 2008) (Fig. 1).

In support of a dual function of FMRP in translational control, there are enhanced protein synthesis rates and synaptic protein levels in *Fmr1* KO mice and mGluR-stimulated translation is absent (Aschrafi et al., 2005; Qin et al., 2005a; Hou et al., 2006; Muddashetty et al., 2007; Zalfa et al., 2007; Liao et al., 2008). Recent work has also observed a deficit in protein synthesis in response to the Gq coupled M1 muscarinic acetylcholine receptors in *Fmr1* KO mice, suggesting a more general role for FMRP in Gq coupled receptor-dependent translation (Volk et al., 2007). Although mGluR5 levels are normal in *Fmr1* KO mice, there is evidence for a reduced association of mGluR5 with constitutive Homer isoforms, a synaptic scaffold and signaling protein (Giuffrida et al., 2005). Consequently, there is reduced localization of mGluR5, at the synapse or postsynaptic density. Importantly, mGluR5-Homer interactions are required for mGluRs to couple to activation of the translational machinery, specifically the PI3 kinase-mTOR (mammalian target of rapamycin) pathway (Ronesi and Huber, 2008b) (Fig. 1). Consistent with an uncoupling of mGluR5 from Homer, mGluRs fail to stimulate PI3K-mTOR in *Fmr1* KO mice. In addition, recent work demonstrates that FMRP interacts with G-protein receptor kinase 2 (GRK2) protein and functions to maintain GRK2 in the cytoplasm (Wang et al., 2008). GRK2 phosphorylates mGluR1 and 5 and may contribute to altered mGluR function in FXS (reviewed in (Mao et al., 2008)). In the dFXR null fly, protein levels of the sole drosophila mGluR (DmGluRA) are elevated suggesting a direct regulation of mGluRs in this organism (Pan et al., 2008). In addition, the enhanced protein synthesis rates in the *Fmr1* KO mouse may be at a ceiling such that *ex vivo* stimulation of mGluRs is ineffective (Todd et al., 2003; Hou et al., 2006; Westmark and Malter, 2007). Future experiments are required to establish the acute function of FMRP in activity-dependent translation as well as determine the effects of constitutive loss of FMRP on the neuronal translation, the latter of which is most relevant for understanding Fragile X Syndrome.

The lack of mGluR-stimulated protein synthesis in *Fmr1* KO mice was difficult to reconcile with the enhanced mGluR-dependent LTD. Because *Fmr1* KO mice have been reported to

have elevated synaptic levels of some proteins known to be synthesized by mGluR activation, it was suggested loss of translational suppression by FMRP lead to an increase in the steady state levels of proteins necessary for mGluR-LTD (dubbed “LTD proteins”) (Zalfa et al., 2003; Hou et al., 2006; Westmark and Malter, 2007; Liao et al., 2008). A direct prediction from this hypothesis is that mGluR-LTD, which is normally blocked by acute administration of protein synthesis inhibitors, should be insensitive to blockade of translation in *Fmr1* KO mice because the proteins necessary for mGluR-LTD expression are present or available at the synapse. In support of this hypothesis, mGluR- and M1 mAChR-induced LTD persists following acute application of protein synthesis inhibitors (Hou et al., 2006; Nosyreva and Huber, 2006; Volk et al., 2007).

Candidate plasticity proteins for LTD

To understand how FMRP regulates protein synthesis dependent synaptic plasticity it was necessary to determine the molecular mechanisms of mGluR-LTD, as well as the identity of the “LTD proteins”. mGluR-LTD is mediated by a rapid endocytosis and persistent decrease in surface expression of postsynaptic ionotropic AMPA receptors (Snyder et al., 2001; Moulton et al., 2006; Zhang et al., 2008). During protein synthesis blockade, mGluRs trigger endocytosis of AMPARs, but AMPARs recycle back to the surface (Snyder et al., 2001; Waung et al., 2008). This result indicates that the newly synthesized “LTD proteins” function to maintain decreased surface AMPAR expression and are likely to regulate AMPAR endocytosis and are translationally suppressed by FMRP (Fig. 1). In support of this model, acute knockdown (KD) of FMRP in cultured rat hippocampal neurons with a small-interfering RNA results in an mGluR5-dependent removal of surface AMPA receptors (Nakamoto et al., 2007).

Recent studies have identified two candidate “LTD proteins” whose mRNAs interact with FMRP and encode proteins known to regulate AMPAR endocytosis. MAP1B is a well characterized dendritic, FMRP interacting mRNA, but its role in mGluR-triggered AMPAR endocytosis has only been recently elucidated (Darnell et al., 2001). mGluR treatment of hippocampal neurons increases MAP1B levels in dendrites, and constitutive knockdown of MAP1B prevents mGluR-induced AMPAR endocytosis (Hou et al., 2006; Davidkova and Carroll, 2007). MAP1b interacts with the GluR2 interacting protein and scaffold GRIP1 and DHPG increases this association (Seog, 2004; Davidkova and Carroll, 2007). Because GRIP1 stabilizes surface GluRs, the synthesis of MAP1b may serve to sequester GRIP1 away from the synapse and destabilize GluR surface expression.

Activity-dependent cytoskeletal associated protein (Arc/Arg3.1) is an abundant dendritic mRNA that interacts with FMRP (Lyford et al., 1995; Zalfa et al., 2003; Iacoangeli et al., 2008). Recent work demonstrated that Arc protein interacts with dynamin 2 and endophilin 3, components of the endocytosis machinery, and functions to increase AMPAR endocytosis rate, making Arc a good candidate for an “LTD protein” (Chowdhury et al., 2006; Rial Verde et al., 2006). Recently, we and Paul Worley’s group independently implicated Arc as an “LTD protein” in mGluR-LTD (Park et al., 2008; Waung et al., 2008). Group 1 mGluRs stimulate rapid synthesis of Arc in dendrites, mGluR-LTD is prevented in Arc KO mice, with Arc knockdown or acute blockade of new Arc synthesis using antisense oligonucleotides. The mechanism by which Arc maintains LTD is thought to be by a persistent (1 hour) increase in AMPAR endocytosis rate, which is caused by mGluR-LTD and requires Arc mRNA translation. Crossing *Fmr1* KO with *Arc* KO mice results in a reduced level of mGluR-LTD (Park et al., 2008). This data suggests that in the absence of FMRP, Arc protein is available to maintain LTD in the absence of new synthesis. Future experiments are required to determine if altered levels or translational control of Arc are responsible for the altered LTD phenotype in *Fmr1* KO mice. If or how enhanced Gq dependent LTD leads to the cognitive symptoms of FXS is unknown. However, because Arc induction is highly linked to plasticity and memory formation

(Plath et al., 2006; Tzingounis and Nicoll, 2006), alterations in Arc-dependent synaptic plasticity in Fragile X Syndrome may provide insight into the altered learning or plasticity-dependent behaviors in the disease.

Widespread Deficits in LTP in Fragile X Syndrome

Another likely contributor to the cognitive deficits in Fragile X Syndrome is the widespread deficit in LTP that is observed in *Fmr1* KO mice. Multiple studies have reported a complete absence of LTP in the neocortex of *Fmr1* KO mice, including visual, prefrontal and somatosensory cortices (Li et al., 2002; Zhao et al., 2005; Desai et al., 2006; Meredith et al., 2007; Wilson and Cox, 2007). Although initial studies demonstrated normal LTP in the hippocampus in *Fmr1* KO mice (Godfraind et al., 1996; Paradee et al., 1999; Li et al., 2002; Larson et al., 2005), more recent work observes a decrease in the magnitude of LTP (Lauterborn et al., 2007; Hu et al., 2008)). Both in the hippocampus and neocortex the deficit in LTP can be overcome by increasing factors involved in LTP induction. For example, in the hippocampus, the lack of LTP is accompanied by a deficit in trafficking of GluR1 containing AMPA receptors and Ras-dependent activation of PI3 kinase, a pathway previously implicated in GluR1 insertion and LTP (Zhu et al., 2002; Qin et al., 2005b). The LTP deficit can be rescued by acute BDNF application or overexpression of upstream activators of PI3K (Lauterborn et al., 2007; Hu et al., 2008) suggesting that BDNF activation of PI3K may contribute its ability to rescue LTP. Interestingly, there appears to be a general deficit in PI3K activation in response to neurotransmitters, including histamine and group 1 mGluRs. This deficit has been suggested to arise from an uncoupling of Ras-to-PI3K or mGluR-Homer-PI3K Enhancer (PIKE) pathways (Hu et al., 2008; Ronesi and Huber, 2008b). Further investigation of the extent of altered PI3K regulation and localization in Fragile X Syndrome, as well as the link to FMRP, may help to determine the mechanisms of the synaptic plasticity phenotypes.

In neocortex, there is a deficit in LTP induced with a protocol that relies on the relative timing of EPSPs and postsynaptic action potentials (APs) or spikes, termed spike-timing dependent plasticity (STDP) (Desai et al., 2006; Meredith et al., 2007). A reduction of L-type Ca^{++} channels and a partial loss of Ca^{++} influx into the spines of layer 2/3 neurons in *Fmr1* KO mice was observed (Meredith et al., 2007). Increasing the number of APs paired with EPSPs during LTP induction increased postsynaptic Ca^{2+} entry into *Fmr1* KO spines and restored STD-LTP in *Fmr1* KO mice. Importantly this work attributed the elongated spine structure to altered Ca^{2+} dynamics in the spine, therefore linking altered spine shape with a functional plasticity deficit. Overall, these studies conclude that LTP expression mechanism is intact in *Fmr1* KO mice. Instead, there is a higher “threshold” for LTP induction that can be overcome by increasing factors during the induction of LTP, such as spine Ca^{2+} influx or activators of PI3K.

Due to the link of mGluRs with FMRP, the mGluR dependence of neocortical plasticity has been investigated in wildtype mice. The observed deficit in LTP in layer 5 neurons of visual cortex was due to a deficit in an mGluR5-dependent LTP (Wilson and Cox, 2007). Interestingly, spike timing dependent-LTD was analyzed in the neocortex of *Fmr1* KO mice and was found to be normal. Although the neocortical LTD relied on mGluR5, it was independent of protein synthesis (Desai et al., 2006). This latter result is consistent with the known function of FMRP and indicates that FMRP regulation of mGluR-dependent LTD may be limited to the LTD paradigms that rely on protein synthesis.

Molecular mechanisms of FMRP-Dependent Synapse Alterations

Translational Control

What is known about the molecular mechanisms by which FMRP alters synapse number or structure? Based upon its canonical function in translational regulation, FMRP likely governs

synaptic function through control of mRNA translation, perhaps locally at synapses or spines. In support of the idea that translational regulatory pathways impact dendritic structure and function, activation of the PI3K→Akt→mTOR pathway (or deletion of endogenous inhibitors), which stimulates cap-dependent translation, causes increased dendritic growth, branching and complexity and causes an increase in dendritic spine number or filopodia-like spines and a decrease in mushroom-shaped spines, a phenotype reminiscent of that seen in *Fmr1* KO mice (Jaworski et al., 2005; Kumar et al., 2005; Tavazoie et al., 2005; Kwon et al., 2006). Conversely, a more direct inhibition of cap-dependent translation by overexpression of eIF4E binding protein, 4EBP, results in decreased dendritic complexity (Jaworski et al., 2005). Another RNA-binding protein, translocated-in-liposarcoma protein (TLS) are localized to dendrites and is trafficked at synapses in an mGluR-dependent manner (Fujii et al., 2005). *TLS*-KO mice display a reduction in mature spines and an increase in filopodia, similar to *Fmr1*-KO mice (Fujii et al., 2005). In addition, loss of the brain-specific, dendritic RNA-binding protein Stauf2 results in an overall decrease in spine and synapse number (Goetze et al., 2006).

Activity of group 1 mGluRs is also associated with changes in spine shape and number. Brief stimulation of group 1 mGluRs causes a translation-dependent increase in spine length (Vanderklish and Edelman, 2002). Genetic reduction of mGluR5 or acute treatment with mGluR antagonists reverses the alterations in dendritic spine number and length in *Fmr1* KO neurons, suggesting that mGluR5 hyperfunction drives the structural spine changes (Dolen et al., 2007; de Vrij et al., 2008). Similarly, some, but not all of the pre- and postsynaptic effects of dFXR1 null fly are rescued by genetic or pharmacological antagonism of the drosophila mGluR, DmGluRA (McBride et al., 2005; Pan and Broadie, 2007; Pan et al., 2008). Thus, in the absence of FMRP, mGluR5-dependent transport and/or translation of critical mRNAs is likely abnormal, leading to an increase in spine length and filopodia number (Dictenberg et al., 2008). Many of the FMRP-interacting mRNAs encode for both pre- and postsynaptic proteins, making it likely that FMRP dependent translational control of a number of synaptic proteins contributes proper synapse development.

Regulation of the Cytoskeleton

FMRP also interacts with mRNAs or proteins that impact the cytoskeleton, a critical determinant of dendritic spine shape. Most evidence for FMRP regulation of the cytoskeleton comes from work in the fly. As mentioned above, dFXR interacts with mRNAs for Futsch (or MAP1b), dRac1, a small Rho GTPase, and Profilin, a regulator of actin dynamics, and suppresses their translation (Zhang et al., 2001; Lee et al., 2003; Reeve et al., 2005). Many of the neurite and synaptic phenotypes in the dFXR null fly are mimicked by overexpression these cytoskeletal regulators or are rescued by the reducing their expression supporting the idea that dFXR may translationally suppresses a program of functionally related proteins for proper neurite and synaptic development. In addition, dFXR and dRac1 interact with a common protein, CYFIP1 (also known as Sra-1; specifically Rac 1 associated protein) and these three genes show interactions in regulation of axon branching and synapse size in the *Drosophila* NMJ (Schenck et al., 2003). Interestingly, because CYFIP only interacts with the GTP-bound or active Rac1, it has been suggested that CYFIP availability may be determined in part by Rac1 activity and in turn regulate dFXR translational function. This mechanism also provides a link between control of the actin cytoskeleton and translation. As mentioned above, mammalian CYFIP1 interacts with FMRP and plays a role in suppression of translation initiation through interactions with eIF4E (Schenck et al., 2001; Napoli et al., 2008). In mammals, CYFIP also interacts with Rac, and Rac-dependent actin remodeling is enhanced in the absence of FMRP or with I304N mutated FMRP expression in mouse fibroblasts (Castets et al., 2005). The latter result suggests that FMRP-RNA interactions or translational control are important for the ability of FMRP to regulate the cytoskeleton. Finally, a downstream

effector of Rac signaling, p21-activated kinase (PAK), is positively coupled with spine number, such that expression of a dominant-negative form of PAK (dnPAK) results in decreased spine density (Hayashi et al., 2004). Notably, introduction of dnPAK into *Fmr1* KO mice led to a wildtype spine phenotype (Hayashi et al., 2007). Thus, the spine phenotype observed in *Fmr1* KO mice bears a striking resemblance to that seen following increased Rac activity, suggesting that this pathway may be particularly important for FMRP regulation of the spine number and morphology through control of the actin cytoskeleton.

Concluding Remarks

Because FXS is caused by loss of function of a single gene, *FMRI*, it presents a valuable opportunity to uncover neurobiological underpinnings of mental retardation and autism. Furthermore, studies of the normal functions of *FMRI* have revealed important roles for dendritic translation in synaptic plasticity and perhaps synapse development. Evidence supports the idea that the primary function of FMRP is to regulate RNA processing, (transport, translation and stability). FMRP binds to a number of dendritically localized mRNAs and/or mRNAs that encode synaptic proteins. Consequently, there are a number of synaptic phenotypes in FXS, as described herein. Future goals include a determination of the primary or acute, cell autonomous functions of FMRP, in contrast to the indirect or compensatory effects of constitutive FMRP loss. This will help to determine how the different synaptic phenotypes are related. For example, do the alterations in synapse development and number cause the synaptic plasticity deficits in *Fmr1* KO mice? Or does FMRP play direct, but multiple roles in both synapse development and plasticity in adults? Despite the large number of FMRP mRNA targets that have been reported, little is known about how their expression and regulation is altered in FXS or if their dysregulation contributes to the FXS phenotype. Although FMRP interacts and likely regulates a number of mRNAs, many of the synapse structure and behavioral deficits are rescued by reduced mGluR5 function. Is the alterations in mGluR5 function caused by loss of the downstream translational suppression function of FMRP or are there more direct alterations in mGluR5 function in FXS? Resolution of these questions will lead to a better understanding of FMRP in normal brain function, how the loss of FMRP leads to mental retardation and autism, and development for better therapies for these prevalent diseases.

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Figure 1. FMRP regulation of mRNA transport and local translation impacts synaptic structure and function

FMRP shuttles to and from the nucleus where it may play a role in nuclear export of mRNAs. FMRP is found both in growth cones, immature axons and mature dendrites, as well as dendritic spines. In these compartments, FMRP is associated with mRNPs and larger RNA granule structures which also contain FMRP-interacting proteins such as FXRs and CYFIP. RNA granules and FMRP travel into dendrites via kinesin motors on microtubules. During transport, it is thought that FMRP functions to translationally suppress cargo mRNAs. Inset: Once in the spine FMRP phosphorylation and ubiquitination are regulated by mGluR activity which is thought to play a role in activation of translation initiation and elongation. Proteins whose translation is regulated by FMRP include Arc and MAP1b, all of which are known to regulate AMPA receptor endocytosis and thereby synaptic function.

Figure 2. Evidence for a direct, postsynaptic role of FMRP in synapse elimination

A Cultured *Fmr1* KO hippocampal neurons (equivalent postnatal day 12–16) have more structural synapses. Top Left, Mixed dissociated culture prepared from both wild-type and *Fmr1*-KO mice labeled with an antibody against FMRP (green fluorescence) and the synaptic marker GluR1 (red fluorescence) (scale 10 μ m). Bottom Left, High resolution image of highlighted sections in top left panel (scale = 5 μ m). Right, Quantification of the number of synaptic puncta on *Fmr1*-KO neurons and neighboring WT neurons for three synaptic markers: surface GluR1, PSD-95, and synapsin. **B**. Acute postsynaptic expression of FMRP results in fewer functional synapses. Top, Depiction of experimental paradigm in which organotypic hippocampal slice cultures are prepared from *Fmr1*-KO mice and biolistically transfected with

GFP-tagged FMRP. Simultaneous whole cell recordings are then obtained from untransfected *Fmr1* KO (FMRP lacking) and FMRP-expressing neurons to quantify electrophysiological changes. Middle, Representative traces for evoked NMDA receptor-mediated synaptic responses (upper left, scale 20 pA, 20 ms), AMPA receptor-mediated synaptic responses (lower left, scale 20 pA, 20 ms), and miniature EPSCs (right, scale 10 pA, 500 ms). Traces from FMRP-expressing (transfected) neurons are shown in grey and those from FMRP-lacking (untransfected) neurons are shown in black. Bottom, Quantification of synaptic function following FMRP expression in *Fmr1*-KO neurons for evoked AMPA receptor-mediated responses, evoked NMDAR-mediated responses, miniature EPSC frequency and amplitude, and synaptic failure rate. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001. Modified with permission from (Pfeiffer and Huber, 2007).

Figure 3. Alterations in synaptic structure, function and plasticity in sensory neocortex of *Fmr1* KO mice

Schematic summarizing major synaptic alterations in sensory neocortex: Size of arrows indicate the magnitude and direction of change. 1) A reduction in the synaptic connectivity of layer (L) 4 excitatory (Ex) neurons with neighboring L4 excitatory neurons, fast spiking (FS) inhibitory interneurons and L2/3 pyramidal neurons. 2) Increase in dendritic spine density and length of layer 2/3 and 5 neurons, although to date no functional change in synapse number has been demonstrated. 3) A reduced threshold for LTP in L2/3 and 5 synapses. 4) Increased L4 axonal length and spread into L2/3. In addition to synaptic changes, increases in intrinsic excitability are observed in L4 excitatory neurons. Overall, there is a circuit hyperexcitability

in response to thalamic stimulation in sensory neocortex which may be mediated by the reduction in excitatory drive onto L4 FS inhibitory neurons, the increase in intrinsic excitability of L4 excitatory neurons and the increase in spine density on pyramidal neurons in L2/3 and 5.