Increasing our Understanding of Human Cognition Through the Study of Fragile X Syndrome

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ABSTRACT: **Fragile X Syndrome (FXS) is considered the most common form of inherited intellectual disability. It is caused by reductions in the expression level or function of a single protein, the Fragile X Mental Retardation Protein (FMRP), a translational regulator which binds to approximately 4% of brain messenger RNAs. Accumulating evidence suggests that FXS is a complex disorder of cognition, involving interactions between genetic and environmental influences, leading to difficulties in acquiring key life skills including motor skills, language, and proper social behaviors. Since many FXS patients also present with one or more features of autism spectrum disorders (ASDs), insights gained from studying the monogenic basis of FXS could pave the way to a greater understanding of underlying features of multigenic ASDs. Here we present an overview of the FXS and FMRP field with the goal of demonstrating how loss of a single protein involved in translational control affects multiple stages of brain development and leads to debilitating consequences on human cognition. We also focus on studies which have rescued or improved FXS symptoms in mice using genetic or therapeutic approaches to reduce protein expression. We end with a brief description of how deficits in translational control are implicated in FXS and certain cases of ASDs, with many recent studies demonstrating that ASDs are likely caused by increases or decreases in the levels of certain key synaptic proteins. The study of FXS and its underlying single genetic cause offers an invaluable opportunity to study how a single gene influences brain development and behavior.** Develop Neurobiol 74: 147–177, 2014

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FRAGILE X SYNDROME—A DISORDER OF COGNITION

Proper cognitive development in humans requires an important balance of nature and nurture that allows for optimal brain circuitry formation and function. The ability of the environment to shape developing brain circuits requires complex cellular and molecular mechanisms that allow neurons to learn, remember, and apply new information gained from interactions with the world. Neurodevelopmental disorders such as Fragile X Syndrome (FXS) and autism spectrum disorders (ASDs), where there is an absence of a single protein or alteration of several proteins (Verkerk et al., 1991; Toro et al., 2010), lead to a delay or failure in the ability of experience to help

pattern connectivity within the brain. Thus, studying these diseases can provide unique insight into the interplay of genetics and the environment in guiding brain development and will likely offer new avenues for brain disease intervention.

Significant attention has been paid to FXS because it is caused by the absence of a single protein, the Fragile X Mental Retardation Protein (FMRP), making FXS the most common monogenic and inherited form of intellectual disability. FXS was first described in 1943 as an X-linked form of intellectual disability (Martin and Bell, 1943). It was later found to be largely caused by the hypermethylation of an expanded CGG trinucleotide repeat (>200 copies) in the 5'-untranslated region of exon 1 of the *Fmr1* gene, leading to transcriptional silencing and loss of the FMRP protein (Verkerk et al., 1991). Missense point mutations and deletions in the *Fmr1* gene coding region have also been found to lead to the development of the disease (De Boulle et al., 1993; Gu et al., 1994; Coffee et al., 2008; Grønskov et al., 2011). FXS affects both males and females and is found at an estimated rate of ~1/2500 in the general population (Hagerman, 2008). Interestingly, random X-inactivation (females) and mosaicism in CGG-size or methylation patterns (males) results in residual and variable levels of FMRP which are strongly correlated with intellectual function (Reiss et al., 1995; Tassone et al., 1999; Loesch et al., 2004; Gothelf et al., 2008). More recently, significant attention has been paid to individuals with the "premutation," a string of 55 to 200 unstable CGG repeats that usually results in transmission of the full mutation to the next generation (Tassone and Hagerman, 2012). These individuals have higher *Fmr1* messenger RNA (mRNA) transcripts but reduced FMRP protein levels (Tassone et al., 2000a,b). Previously, individuals harboring this premutation were thought to be asymptomatic; however, more careful examinations have shown that these individuals show cognitive and behavioral impairments, including deficits with attention, inhibitory control and working memory, as well as alterations in emotional states such as anxiety, depression and hostility (De Rubeis et al., 2012). These phenotypes appear to be correlated with the number of CGG repeats and levels of FMRP protein, pointing to a possible spectrum of severity with increasing number of repeats and reduced FMRP levels (Cornish et al., 2009; Mínguez et al., 2009; Hessl et al., 2011). The premutation is also associated with cognitive decline, dementia, and Parkinsonism in males over the age of 50 (called Fragile X-associated tremor/ataxia syndrome), as well as infertility, early menopause, and ovarian problems in women (called Fragile X-associated primary ovarian insufficiency), possibly due to mRNA toxicity and a build-up of intranuclear ubiquitin-positive inclusions (Tassone and Hagerman, 2012; De Rubeis et al., 2012). Interestingly, an extremely severe form of FXS is caused by a single amino acid substitution (I304N) (De Boulle et al., 1993), suggesting an important link between this region of the protein and the disorder and pointing to the importance of studying the structure of the FMRP protein in order to gain clues about its function. Therefore, tight regulation of the levels of both *Fmr1* mRNA and FMRP is required for proper cognitive function.

Accumulating evidence suggests that the diverse symptoms of FXS are likely due to an interplay between genetic factors (e.g. loss of FMRP, inherited individual differences) and environmental factors (e.g. upbringing, life experience, schooling, behavioral enrichment), all of which contribute to the proper development and remodeling of neural circuitry necessary for cognition. For instance, compared with normal children, FXS children show greatly reduced or altered interactions with their environment. This appears to be caused by an increase in their sensitivity to sensory stimuli (Baranek et al., 2008). Indeed, males diagnosed with FXS have increased baseline and stress-induced levels of cortisol, elevated heart rates and reduced parasympathetic tone compared with unaffected children, all indicative of increased physiological arousal and heightened emotional responses to their environment (Hessl et al., 2004; Baranek et al., 2008; Bailey et al., 2011). This hyperawareness of the environment leads these children, at an early age, to avoid sensory experiences and social interactions—experiences that help reinforce the development of skills required to properly process and interact with the world (Roberts et al., 2009; Bailey et al., 2011). This early avoidance likely contributes to the delay in acquiring basic sensorimotor skills and to a range of cognitive disabilities including, but not limited to, problems with attention and impulse control, stereotypic and perseverative language and motor behaviors, and poor visual-spatial memory that persist into adolescence and adulthood (Bailey et al., 2011; Bray et al., 2011; Smith et al., 2012). Thus, in addition to deficits in neuronal circuit hard-wiring directly caused by the loss of FMRP, important environmental interactions that are needed to appropriately sculpt brain circuits during early development are diminished in children with FXS.

Recent research has suggested a significant overlap between FXS and ASDs. ASDs are found at a very high rate in the general population (1/88) and are characterized by abnormal social interactions,

repetitive behaviors, limited interests and language problems (CDC, 2012). Like FXS, ASDs are characterized by hypersensitivity to the environment and avoidance of novel stimuli (O'Neill and Jones, 1997; Gerlai and Gerlai, 2004; Iarocci and McDonald, 2006; Kern et al., 2007), potentially contributing to the difficulty these children face in learning new skills and behaviors. Children who present with FXS and ASD have significantly worse behavioral outcomes than children with FXS alone, showing greater deficits in the areas of language, communication and social behaviors (Bailey et al., 2011). Indeed, approximately 30 to 60% of FXS patients present with features of ASD (Rogers et al., 2001; Kaufmann et al., 2004) and 5% of identified ASD cases are found to be due to hypermethylation of the *Fmr1* gene, making loss of FMRP a leading single gene cause of autism (Bassell and Warren, 2008; Pfeiffer and Huber, 2009; Hagerman et al., 2010; Budimirovic and Kaufmann, 2011).

A characteristic feature of both FXS and ASD is an abnormal increase in brain weight and head circumference compared with normal children over the course of the first two years of life (Bailey et al., 2011). Studies have also demonstrated alterations in structure and/or function in brain nuclei making up the limbic system, including the hippocampus, amygdala, caudate nucleus and prefrontal cortex in children with either FXS and/or ASD (Reiss et al., 1995; Eliez et al., 2001; Hessl et al., 2004; Menon et al., 2004; Schumann et al., 2004). Structural and functional changes in these brain areas point to potential abnormalities in the genetic hard-wiring and/or experience-dependent remodeling of these neuronal circuits, either through changes in the rates of cell division or cell death, or changes in the cellular and molecular mechanisms that ensure the proper strengthening and pruning of neuronal connections. Since the limbic system is involved in the reinforcement of new behaviors and appropriate behavior selection (Hessl et al., 2004), the inability to properly establish and reorganize the underlying neuronal circuits in response to experience may lead to the common behavioral abnormalities seen in both disorders. Since ASDs encompass a large number of variable cognitive and behavioral deficits, most likely resulting from a plethora of different genetic and environmental factors (Miles, 2011), FXS offers an alternative model to study the relationship between a single gene and its influence on the brain and behavior.

FMRP—Structure and General Function

The human *Fmr1* gene is composed of 17 exons and spans about 38kb in the Xq27.3 region of the X-chromosome (Verkerk et al., 1991). The *Fmr1* gene encodes the FMRP protein, a cytoplasmic RNA-binding protein (Devys et al., 1993; Verheij et al., 1993). Along with FMRP there are two human paralogs, Fragile X-Related Protein 1 (FXR1P) and Fragile X-Related Protein 2 (FXR2P), which share over 60% amino acid identity with FMRP (Tamanini et al., 1997; Khandjian et al., 1998). The *Fxr1* gene maps to chromosome 3q28, whereas *Fxr2* maps to 17p13.1 and they are both autosomal genes coding for RNA-binding proteins (Khandjian, 1999). The Fragile X protein family is highly conserved in sequence and structure throughout evolution and is present in both vertebrates and invertebrates, with at least one ortholog identified in mouse, chicken, fly, frog, zebrafish and aplysia (Ashley et al., 1993a; Price et al., 1996; Wan et al., 2000; Tucker et al., 2004; Blonden et al., 2005; van't Padje et al., 2005). Intriguingly, an ortholog was also discovered in the cnidarian hydroid *Hydractinia echinata*, one of the oldest living animals possessing a nervous system, but not in pre-nervous system organisms, suggesting that the Fragile X gene arose in the common ancestor of the cnidarians and bilaterians and may have played an important role in the development of primitive nervous systems (Guduric-Fuchs et al., 2004). Since only a single ortholog of the Fragile X family exists in invertebrates and tunicates, the three members of the Fragile X family may have evolved through two successive gene duplication events in the vertebrate ancestor, after the divergence of the tunicates (Kirkpatrick et al., 2001; Guduric-Fuchs et al., 2004).

This strong evolutionary conservation in sequence and structure has given researchers insights into FMRP's potential functions. The highest degree of conservation is seen in the RNA-binding domains of the protein, pointing to the importance of these domains to the function of FMRP (Ashley et al., 1993a; Price et al., 1996; Wan et al., 2000; Blonden et al., 2005). More specifically, the domain structure of the FMRP protein consists of four RNA-binding motifs: a cluster of arginine and glycine residues (RGG box), two ribonucleoprotein K homology domains (KH domains) and an RNA-binding domain in the N-terminal region of the protein (Siomi et al., 1994; Adinolfi et al., 1999a,b, 2003; Zalfa and Bagni, 2004). Initial *in vitro* studies showed that the RGG motif of the FMRP protein binds to a class of mRNAs with a characteristic tertiary structure, named G-quartets (Darnell et al., 2001). However, *in vivo* studies revealed that only a minority of FMRP bound mRNAs contained G-quartets, suggesting that this motif is not the sole determinant of FMRP binding (Brown et al., 2001). Along these lines of evidence,

Darnell et al. (2005) found that the second KH domain of FMRP specifically binds to RNA complexes featuring a "loop-loop" pseudoknot tertiary structure termed a "kissing complex." A recent *in vivo* extension of this work, using high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP), unexpectedly found that polyribosome-associated FMRP binds all along the open reading frame of its target mRNAs (Darnell et al., 2011). In addition, a recent study has revealed several distinct RNA recognition elements in both the coding sequence and 3' untranslated region of FMRP target mRNAs (ACUK and WGGA) (Ascano et al., 2012). This binding to specific sequences and/or specific tertiary structures on target mRNAs points to a role for FMRP in some aspect of mRNA metabolism (Doyle and Kiebler, 2011). Indeed, early studies in cell culture found that FMRP interacts with ribosomal subunits and associates with actively translating polyribosomes in ribonucleoprotein particles (RNPs), pointing to a role for FMRP in translational control (see later section for more details) (Khandjian et al., 1996; Tamanini et al., 1996; Siomi et al., 1996; Corbin et al., 1997). Interestingly, FMRP with a missense mutation in the KH2 RNA-binding domain (I304N), which causes severe mental retardation in humans, displays reduced affinity for kissing-complex and ACUK-containing mRNAs and fails to bind its target mRNAs and incorporate into polyribosomes *in vivo* (De Boulle et al., 1993; Feng et al., 1997a; Zang et al., 2009; Ascano et al., 2012).

FMRP undergoes multiple post-translational modifications that affect not only its ability to bind to mRNAs and protein partners but also its function. One of these modifications includes arginine methylation of the RGG box (Blackwell et al., 2010). In general, arginine methylation affects protein-RNA interaction, protein-protein interactions and protein localization (Gary and Clarke, 1998). *In vitro* research done on FMRP has demonstrated that this methylation reduces the ability of FMRP to bind G-quartet containing RNAs (Stetler et al., 2006; Dolzhanskaya et al., 2006a,b; Blackwell et al., 2010). Moreover, these methylated arginines are important for modulating FMRP polyribosome association (Blackwell et al., 2010). Research has also shown that the phosphorylation status of FMRP can affect its translational control activity (Ceman et al., 2003). FMRP from the murine brain and cultured cells is phosphorylated between residues 483 and 521, N-terminal to the RGG box. Primary phosphorylation occurs on the highly conserved serine 499 which, in turn, triggers hierarchal phosphorylation of nearby serines. Ribosomal run-off assays have demonstrated an association between phosphorylated FMRP and stalled ribosomes, whereas non-phosphorylated FMRP associates with actively translating ribosomes (Ceman et al., 2003). These studies suggest a dynamic regulation of FMRP function and point to potential context-dependent influences on its ability to regulate mRNA metabolism.

In addition to its RNA-binding domains, FMRP also contains a nuclear localization signal (NLS) and a nuclear export signal (NES), indicating that the protein has the capability to act as a chaperone for trafficking mRNAs from the nucleus into the cytoplasm (Eberhart et al., 1996). One complication to studying the properties of FMRP is the prediction that extensive alternative splicing of the mammalian *Fmr1* mRNA could produce up to 48 distinct mature transcripts, although the biological presence of all these transcripts has not been confirmed (Ashley et al., 1993b; Verkerk et al., 1993; Khandjian et al., 1995; Khandjian, 1999; Evans et al., 2012).

FMRP is also involved in a number of protein-protein interactions which may act to modify its affinity for certain target mRNAs and/or its function. Many of these protein binding partners are also RNA-binding proteins or cytoskeleton-associated proteins (Bardoni et al., 2006). Specifically, FXR1P, FXR2P, NUFIP1 (nuclear FMRP interacting protein 1) and 82-FIP (82 kDa FMRP-interacting protein) are all RNA-binding proteins which interact with FMRP through its N-terminal domain (Bardoni et al., 2006). Cytoplasmic FMRP interacting protein-1 (CYFIP1) and CYFIP2, which also interact with FMRP via its N-terminus, may act to link FMRP to the Rho GTPase signaling pathway and actin cytoskeleton remodeling (Schenck et al., 2001). Finally, FMRP's C-terminal domain is involved in interactions with microspheruleprotein 58 (MSP-58), KifC3, Ran, BPM, and SMN (survival of motor neuron) (Menon et al., 2004; Bardoni et al., 2006; Davidovic et al., 2006, 2007; Piazzon et al., 2008). The latest identified FMRP protein binding partner is Caprin1, an RNA-binding protein which acts as a translational repressor in neurons (Shiina et al., 2005). Caprin1 binds directly to FMRP between amino acids 422 to 439 which is also where the NES domain of FMRP is found (El Fatimy et al., 2012). The data suggest that FMRP can act at two different levels: (1) it can interact directly with target RNAs and (2) it can also act as a protein adaptor by interacting with different RNA-binding proteins such as Caprin 1 (El Fatimy et al., 2012). How FMRP's affinity for its mRNA targets or function is modified in the presence of each of these interacting proteins remains to be determined.

Similar to FMRP, FXR1P, and FXR2P contain characteristic sequence motifs including an RGG box

and two KH domains as well as NLS and NES signals (Khandjian, 1999). Moreover, the two paralogs also associate with RNPs found in translating ribosomes (Corbin et al., 1997; Khandjian et al., 1998; Cook et al., 2011). *In vitro* and *in vivo* evidence indicates that these proteins can form homo- and heteromers with each other, suggesting potential interdependency among these proteins for their function (Zhang et al., 1995; Tamanini et al., 1997). However, despite their important roles in muscle and brain development, much less is known about FXR1P and FXR2P in comparison to FMRP (Bontekoe et al., 2002; Mientjes et al., 2004).

Spatial and Temporal Expression Pattern of FMRP and its Paralogs FXR1P and FXR2P

The severe impact of FXS on cognitive function is not surprising when one looks at the expression pattern of FMRP during development and across different brain regions. During development, FMRP shows a fairly ubiquitous distribution in body tissues including brain, muscle tissue, and internal organs of mice. However, in the adult, FMRP is enriched in the brain and testes of mice and becomes much less abundant in muscle tissue (Khandjian et al., 1995). Expression is mostly confined to neuronal cells in the adult brain (Tamanini et al., 1997; Bakker et al., 2000), although FMRP expression has also been identified in developing glial cells (Pacey and Doering, 2007). In contrast, FXR1P expression is relatively high in heart and skeletal muscle tissue with much lower expression in the brain and testes (Tamanini et al., 1997; Khandjian et al., 1998; Bakker et al., 2000). On the other hand, FXR2P is expressed at higher levels in the brain of fetal and adult mice with lower levels of expression observed in the liver, heart and skeletal muscle tissue (Bakker et al., 2000). Interestingly, three-dimensional mapping techniques in monkeys have revealed that FMRP expression is especially high in the cerebellum, striatum and temporal lobe (Zangenehpour et al., 2009). Temporal lobe structures, especially the hippocampus, play an important role in mediating memory and learning processes. This suggests that deficits in behavior and cognition in FXS patients may be linked to the loss of FMRP from specific sub-regions of the brain (Zangenehpour et al., 2009).

Immunohistochemical studies have explored the spatial and temporal expression of FMRP, FXR1P, and FXR2P in the mammalian brain (Tamanini et al., 1997; Bakker et al., 2000). All three proteins are highly expressed in the cytoplasm of many types of fetal and adult neurons, including cerebellar Purkinje cells, brainstem, and cortical neurons (Tamanini et al., 1997; Bakker et al., 2000). FXR1P also exhibits a strong nuclear localization in the fetal, but not adult, human brain (Tamanini et al., 1997). Looking more closely at the subcellular distribution of these three proteins using cultured mouse hippocampal neurons, FMRP, FXR1P, and FXR2P all exhibit a strong expression in the cytoplasm and proximal dendrites, with little expression in distal dendrites (Tamanini et al., 1997; Cook et al., 2011). Thus, the three homologs exhibit largely overlapping cytoplasmic and dendritic expression patterns in developing and adult mammalian neurons. This suggests that Fragile X proteins control some aspect of mRNA metabolism in the somatodendritic compartment.

In addition to their high expression levels in dendrites, Fragile X proteins have also been identified in axons (Antar et al., 2006; Price et al., 2006; Centonze et al., 2008; Christie et al., 2009; Akins et al., 2012). More specifically, Christie et al. (2009) characterized and defined a novel Fragile X protein-containing axonal structure called the Fragile X Granule (FXG). FXGs are localized in developing axonal fiber tracts and in terminal fields (Akins et al., 2012) as well as in axonal and presynaptic compartments of restricted circuits (Christie et al., 2009). Notably, FXGs are found in abundance in sensory and motor processing areas including the thalamus, motor cortex, hippocampus, olfactory bulb and brainstem (Akins et al., 2012). These results suggest that Fragile X Proteins also play an important role in the presynaptic compartment and that the myriad behavioral deficits seen in FXS may be explained by loss of FMRP from a large number of important neuronal circuits.

Control of Local Protein Synthesis via FMRP

Based upon the structure, function and expression pattern of FMRP and the fact that FMRP has been shown to bind to the mRNAs of important synaptic and cytoskeletal proteins such as CaMKIIα, Arc, MAP1b, and PSD95 (Zhang et al., 2001; Zalfa et al., 2003; Muddashetty et al., 2007), it has been hypothesized that one of the main functions of this molecule is to control some aspect of RNA metabolism in parts of the brain important for cognition. Further research looking at its subcellular distribution has shown that FMRP is found in dendrites and dendritic spines (Feng et al., 1997a; Weiler et al., 1997; Antar et al., 2004; Ferrari et al., 2007), as well as in axonal growth cones, axons and presynaptic terminals (Antar et al., 2006; Price et al., 2006; Centonze et al., 2008;

Christie et al., 2009; Akins et al., 2012), suggestive of a role for FMRP in the local control of RNA metabolism in multiple subcellular compartments of neurons.

Much attention has been given to the role of FMRP in regulating mRNAs important for synaptic function, as direct translational control over a pool of synaptic mRNAs may provide the impetus for rapid, synapse-specific insertion of new synaptic proteins in response to synaptic activity (Darnell et al., 2011). Studies in the 1960s provided the first evidence that synaptic fractions (containing both pre- and postsynaptic compartments) were capable of incorporating radioactive amino acids, demonstrating the presence of functional protein synthesis machinery at synapses (Bodian, 1965; Autilio et al., 1968; Morgan and Austin, 1968). However, it was not until the 1980s that further research would corroborate and extend these findings. Using electron microscopy techniques, polyribosomes, the major workhorses of the translation machinery, were found to be distributed throughout the dendrite and at a subset of dendritic spines in the hippocampus (Levy et al., 1982; Steward, 1983; Steward and Falk, 1985; Steward and Ribak, 1986; Steward and Reeves, 1988). Recent microarray studies have shown that approximately 400 distinct mRNAs are constitutively, although not exclusively, localized to dendrites (Miyashiro et al., 1994; Eberwine et al., 2002; Zhong et al., 2006). In addition, several mRNAs encoding components of the synaptic vesicle have been detected in axons (Akins et al., 2009). Research has shown that local protein synthesis from pre-existing mRNAs plays an important role in certain forms of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), processes that are thought to govern experience-dependent remodeling of synapses in the brain (Frey et al., 1989; Kang and Schuman, 1995; Huber et al., 2000; Yin et al., 2006). Local protein synthesis in axons is required for growth cone turning and collapse (Campbell and Holt, 2001; Wu et al., 2005; Leung et al., 2006) as well as synapse-specific long-term facilitation at Aplysia sensory-motor synapses (Martin et al., 1997). Therefore, an increase in local protein synthesis at active synapses is one mechanism through which the environment/experience (via changes in neuronal activity at specific synapses) can adjust the properties of a genetically hard-wired neuronal circuit.

The experience-dependent strengthening and elimination of specific neuronal connections requires tight regulation of local protein synthesis. This tight regulation is provided by a plethora of RNA-binding proteins and signaling pathways which couple synaptic activity to increased protein synthesis (Kiebler and Bassell, 2006; Bhakar et al., 2012). Each mRNA exits the nucleus bound by a specific complement of RNA-binding proteins, forming an RNP. Together, these RNA-binding proteins are involved in the transport, stability, storage, translational repression and activity-dependent translational derepression/activation of these mRNAs, ensuring that new proteins are made and inserted into synapses only when and where they are needed (Doyle and Kiebler, 2011). RNPs are known to be heterogeneous with different compositions and functions. Broadly speaking, RNPs can be categorized into RNA transport particles, stress granules, P-bodies, and RNA granules (Sossin and DesGroseillers, 2006). RNA transport particles and RNA granules are involved in the transport and storage of translationally repressed mRNAs. Stress granules and P-bodies represent storage and/or degradation sites of mRNAs (Sossin and DesGroseillers, 2006; Kedersha and Anderson, 2007). This heterogeneity in RNP composition and structure allows for the compartmentalization of RNA metabolism within the dendrite and synapses.

Surprisingly, FMRP has been localized in all four types of RNPs, as well as a newly-identified pre-synaptic FXG, indicative of a role for FMRP in multiple aspects of dendritic and axonal mRNA transport and metabolism (Mazroui et al., 2002; Antar et al., 2005; Aschrafi et al., 2005; Barbee et al., 2006; Cheever and Ceman, 2009b). Since FMRP binds to approximately 800 brain mRNAs, many of which have been found to be associated with ASDs and synaptic plasticity (Ashley et al., 1993a; Brown et al., 2001; Darnell et al., 2011; Ascano et al., 2012), it could take on multiple roles depending on the particular mRNA target, cell type or developmental context.

RNA Stability. Several studies have provided evidence that FMRP may, either directly or indirectly, stabilize certain mRNAs. Loss of FMRP in mouse brain results in reduced levels of proteins important for mRNA translation and synaptic function, including ribosomal component p40/LRP, G protein coupled receptor kinase 4 (GRK4), dystroglycan (DAG1) and several $GABA_A$ receptor subunit mRNAs (Miyashiro et al., 2003; Gantois et al., 2006; D'Hulst et al., 2006). Zalfa et al. (2007) were the first to demonstrate that FMRP can stabilize brain mRNAs. They found that FMRP stabilizes PSD95 mRNA in the hippocampus, but not cortex, and that this effect is mediated through direct binding of FMRP to its 3' untranslated region (Zalfa et al., 2007; De Rubeis and Bagni, 2010). This study

provided the first example of a region-specific effect of FMRP on mRNA stability.

RNA Transport. FMRP is known to shuttle into and out of the nucleus through its NLS and NES sequences, respectively, and is co-transcriptionally bound to its cargo pre-mRNAs in the nucleus (Feng et al., 1997b; Kim et al., 2009), suggestive of a role for FMRP in mRNA transport. However, while FMRP traffics with its target mRNAs from the cell body into dendrites and axons (Antar et al., 2004, 2005, 2006; De Diego Otero et al., 2002), it is not actually required to maintain the steady-state levels nor constitutive localization of many of its mRNA targets in dendrites, including MAP2, CaMKIIα, RGS5, SAPAP4 and the $GABA_A$ receptor δ (Steward et al., 1998a; Muddashetty et al., 2007; Zalfa et al., 2007; Dictenberg et al., 2008). Loss of FMRP, however, impairs the activity-dependent transport of these mRNAs into dendrites by reducing their interaction with kinesin-1 (Dictenberg et al., 2008). FMRP also regulates the movement of FMRP target mRNAs in *Drosophila* neurons, supporting the idea that FMRP influences mRNA transport by acting as an adaptor protein between its target mRNAs and microtubule-based motors (Estes et al., 2008).

RNA Storage and Anchoring at Synapses. Paradoxically, when looking at the movement of fluorescently-tagged RNA-binding proteins or mRNAs in cultured neurons, the majority of RNPs, usually of unknown composition, are immobile (Ainger et al., 1993; Knowles et al., 1996; Köhrmann et al., 1999; Elvira et al., 2006; Miller et al., 2009). This holds true for FMRP-containing RNPs as well, which are often located at the base of actin-rich filopodia and dendritic spines (Antar et al., 2004, 2006; Barbee et al., 2006). This suggests that a large fraction of FMRP (as well as other RNA-binding proteins) may actually help to store and anchor mRNAs to the cytoskeleton. This is further supported by fluorescent recovery after photobleaching experiments that show rapid exchange between cytoplasmic FMRP molecules and large RNPs, leading us to speculate that transport RNPs, falling below the level of detection of live imaging, replenish these more stable stores of RNPs (Antar et al., 2004; Barbee et al., 2006). Experiments in non-neuronal cells have shown that mRNAs are locally anchored in specific subdomains of the cell via interactions with the actin cytoskeleton or microtubules (Mili et al., 2008; Singer, 1992). Interestingly, Mili et al. (2008) identified FMRP as part of a complex which locally anchors a diverse group of mRNAs to microtubules in migrating mouse fibroblasts. FMRP also interacts directly with the actin-associated protein CYFIP1 in brain (Schenck et al., 2001). Further investigation is needed to determine whether this interaction helps anchor FMRP and its target mRNAs to the actin or microtubule cytoskeleton in neurons.

RNA Translational Control. New protein synthesis from an mRNA begins with the binding of the initiation factor eIF2 to GTP and Met-$tRNA_i^{Met}$ to form a ternary complex which then associates with the small 40S ribosomal subunit to form the 43S pre-initiation complex. The 43S complex is then guided to 5' capped mRNAs, which make up the majority of nuclear-transcribed mRNAs, by interaction with the cap-binding complex eIF4F (made up of the cap-binding protein eIF4E, the RNA helicase eIF4A and eIF4G) (Costa-Mattioli et al., 2009). The complex then scans the mRNA until it reaches the initiation codon, at which point it is joined by the 60S complex, a complex composed of the large ribosomal subunit and its associated translation factors. Translation elongation proceeds with the help of elongation factors, such as eEF2. Translation termination occurs when the ribosome reaches the stop codon and is released from the mRNA with the help of termination factors (Costa-Mattioli et al., 2009).

The rate-limiting and major regulatory step in mRNA translation is at the point of initiation. This step is regulated by 4E-BPs which bind to eIF4E and prevent joining of eIF4G and the small ribosome to the mRNA, thereby blocking initiation. Hyperphosphorylation of the 4E-BPs causes them to dissociate from eIF4E, allowing initiation from capped mRNAs to occur. Other points of regulation which are less well understood include phosphorylation of eIF4E and the small ribosomal subunit S6 (Kelleher et al., 2004a). S6, eIF4E and 4E-BP phosphorylation are under the control of both the ERK-MAPK and PI3K-mTOR pathways, which are known to play important roles in local protein synthesis, synaptic plasticity and learning and memory (Tang et al., 2002; Gallagher et al., 2004; Hou and Klann, 2004; Kelleher et al., 2004b). Both of these pathways are coupled to synaptic activity via group 1 metabotropic glutamate receptor (Gp1-mGluR) signaling (Ferraguti et al., 1999; Gallagher et al., 2004; Hou and Klann, 2004; Banko et al., 2006; Antion et al., 2008; Ronesi and Huber, 2008; Sharma et al., 2010).

Another key point of control is the phosphorylation status of eIF2α. Phosphorylation of eIF2α prevents the ternary complex from forming and therefore halts translation initiation. The phosphorylation status of eIF2α is controlled by four kinases, GCN2, PERK,

PKR, and HRI, with each kinase activated in response to different types of cellular stresses (Costa-Mattioli et al., 2009). Reductions in the levels of eIF2α phosphorylation reduce the threshold for long-lasting synaptic plasticity and facilitate long-term memory formation (Costa-Mattioli et al., 2005, 2007). How synaptic activity couples to eIF2α phosphorylation is currently unknown. Although not generally considered a rate-limiting step, mRNA translation can also be regulated at elongation (Olsen and Ambros, 1999; Clark et al., 2000; Hussey et al., 2011). In fact, converging evidence suggests that inhibition of translational elongation may be a general mechanism used by neurons to ensure rapid new protein synthesis of specific subsets of mRNAs in response to patterned synaptic activity (Scheetz and Nairn, 2000; Park et al., 2008; Costa-Mattioli et al., 2009).

By far the best-characterized function of FMRP is as a translational regulator. Based on initial *in vitro* translation assays, FMRP was first proposed to function as a repressor of mRNA translation (Laggerbauer et al., 2001; Li et al., 2001). *In vivo* support for this proposal came from the finding that the fly FMRP homolog, dFMRP, binds to and represses futsch mRNA (MAP1B) (Zhang et al., 2001). This was further corroborated by evidence demonstrating increased protein synthesis, as well as, increased association of dendritic mRNAs with translating polyribosomes in *Fmr1* knockout mice (Qin et al., 2005; Hou et al., 2006; Dölen et al., 2007; Muddashetty et al., 2007; Osterweil et al., 2010). In addition, *Fmr1* knockout mice display enhanced, protein synthesis-independent, mGluR-LTD, a form of synaptic plasticity that normally relies on rapid new protein synthesis (Weiler and Greenough, 1993; Huber et al., 2000, 2002). This suggests that loss of FMRP leads to a basal increase in the expression of proteins required for long-term depression ("LTD proteins"). Together, these results led to the mGluR hypothesis of FXS which proposes that loss of FMRP leads to an upregulation of genes normally translated in response to mGluR signaling (Bhakar et al., 2012).

However, these results are not easily reconciled with studies showing biochemical fractionation of FMRP with actively translating polyribosomes (Eberhart et al., 1996; Khandjian et al., 1996; Tamanini et al., 1996; Corbin et al., 1997; Feng et al., 1997a; Khandjian et al., 2004; Stefani et al., 2004). In addition, synaptoneurosomes prepared from *Fmr1* knockout mice did not show the expected increase in protein synthesis in response to group 1 mGluR activation (Todd et al., 2003; Weiler et al., 2004; Hou et al., 2006). Recently, FMRP has actually been shown to activate the translation of several target genes (Bechara et al., 2009; Fähling et al., 2009). Based on these results FMRP may be functioning in two ways: (1) acting as either a repressor or an activator of translation, depending on the target mRNA and/or (2) repressing and activating the translation of its target mRNAs, thereby acting as a "translational switch" in response to synaptic activity (Bassell and Warren, 2008). The latter hypothesis has been supported by several studies. First, FMRP associates with both stalled polyribosomes (phosphorylated form) and actively translating polyribosomes (dephosphorylated form), depending on its phosphorylation status (Ceman et al., 2003). Second, neuronal activity controls the segregation of FMRP between polyribosome-free, translationally inactive transport RNPs and polyribosome-containing RNPs (Wang et al., 2008). Third, only a form of FMRP that can be dephosphorylated is capable of playing a role in synapse elimination (Pfeiffer and Huber, 2007). Together, these studies suggest that FMRP function depends on an activity-dependent switch based on its phosphorylation status.

The ability of FMRP to toggle its function likely has multiple points of regulation. FMRP phosphorylation depends on a balance between the activities of S6 kinase-1 (S6K1) and protein phosphatase 2A (PP2A), both of which are activated downstream of the mGluR-mTOR and mGluR-ERK signaling pathways (Mao et al., 2005a,b; Narayanan et al., 2007; Narayanan et al., 2008). Further evidence suggests that mGluR-LTD leads to a rapid, transient increase in FMRP, which is then degraded by the proteasome (Weiler et al., 1997; Hou et al., 2006), indicating the presence of complex feedback loops that control FMRP levels and function. A more recent study has shown that dephosphorylation of FMRP targets it for ubiquitination and degradation by the ubiquitin-proteasome pathway (Nalavadi et al., 2012). Therefore, FMRP function appears to be regulated both by phosphorylation/dephosphorylation and by synthesis/degradation, potentially allowing for a high degree of fine-tuning of FMRP activity.

Recently, Niere et al. (2012) provided an elegant description of this "switch" capability of FMRP. They focused their attention on the control of Arc mRNA translation, one of FMRP's target mRNAs (Zalfa et al., 2003; Park et al., 2008). Arc is an immediately-early gene whose mRNA is rapidly transcribed, transported and translated in neuronal dendrites in response to salient experience and/or mGluR stimulation (Lyford et al., 1995; Steward et al., 1988b; Park et al., 2008; Waung et al., 2008). Although basal dendritic Arc protein levels and

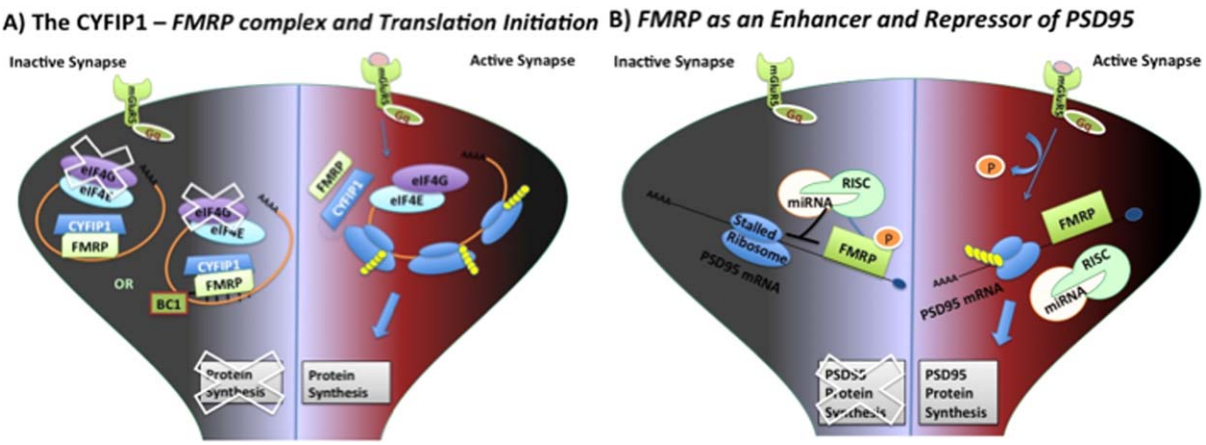

Figure 1 A) The CYFIP1-FMRP or CYFIP1-FMRP-BC1 complex is one of the proposed models for mRNA translational repression and activation. During basal conditions FMRP binds CYFIP1, and blocks translation initiation for its target mRNAs. However, under synaptic activity the CYFIP1- FMRP complex is displaced from the cap-binding protein eIF4E, leading to ribosome recruitment and translation initiation. B) FMRP acts both as an enhancer and as a repressor of PSD95. Under basal conditions phosphorylated FMRP forms an inhibitory complex with miRNA-125 and RISC to inhibit PSD95 translation. Upon mGluR signaling FMRP is dephosphorylated and released from the miRNA-125–RISC complex which results in the translation of PSD95 mRNA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mGluR-LTD are enhanced in *Fmr1* knockout neurons, rapid mGluR-dependent translation of Arc requires FMRP (Niere et al., 2012). This rapid translation of Arc requires mGluR-dependent activation of PP2A and dephosphorylation of FMRP (Niere et al., 2012). This study provides added evidence that, in addition to serving as a repressor of mRNA translation, FMRP also facilitates mRNA translation in an activity-dependent manner. Since Arc expression leads to synapse remodeling via the internalization of AMPA receptors and is required for experience-dependent synaptic and behavioral plasticity (Chowdhury et al., 2006; Bramham et al., 2010), misregulation of Arc mRNA translation may be related to the synaptic and cognitive deficits seen in FXS.

Mechanisms of Translational Control. Several recent articles have shed light on the potential molecular mechanisms through which FMRP represses its target mRNAs. In addition to being found with heavy polyribosome fractions, researchers have found evidence for FMRP in light mRNP fractions containing monomeric 80S ribosomes, translation initiation factors and a small, non-coding RNA called brain cytoplasmic RNA 1 (BC1) (Zalfa et al., 2003, 2005; Gabus et al., 2004; Johnson et al., 2006; Napoli et al., 2008; Centonze et al., 2008; but see Wang et al., 2005; Iacoangeli et al., 2008), suggestive of a role for FMRP in controlling the initiation of translation. In support of this idea, FMRP has been shown to block translation initiation by binding to the novel 4E-BP, CYFIP1/Sra1 (Napoli et al., 2008). Synaptic activity displaces CYFIP1 from eIF4E, leading to ribosome recruitment and translation initiation (Napoli et al., 2008) (Fig. 1). However, Darnell et al. (2011) put forth a different mechanism when they surprisingly found, using high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP), that FMRP binds not only to the 3' and 5' untranslated regions of mRNAs, but also all along their open reading frames. Using polyribosome profile analyses, they suggest that FMRP represses translation by stalling ribosomes along the mRNA, a mechanism which supports results from previous publications (Ceman et al., 2003; Pfeiffer and Huber, 2007; Darnell et al., 2011). As explained previously, this translational repression may be alleviated by activity-dependent dephosphorylation of FMRP, which is thought to convert FMRP-bound stalled ribosomes into actively translating polyribosomes (Ceman et al., 2003; Niere et al., 2012). Since FMRP associates with both ribosome-containing and ribosome-free RNPs, it is possible that FMRP has the capability to inhibit both translation initiation and elongation (Wang et al., 2008).

miRNAs, small non-coding RNAs which bind to mRNAs in a sequence-dependent manner, also play important roles in controlling the translation of specific subsets of mRNAs important for synaptic plasticity. The RNA-induced silencing complex (RISC),

which includes miRNAs bound by Dicer and argonaute proteins, normally functions to repress or degrade target mRNAs (Nilsen, 2007; Vasudevan and Steitz, 2007; Fabian et al., 2011). In addition to, or in combination with, the mechanisms described above, FMRP also functions with the miRNA-RISC pathway. Several studies have demonstrated that FMRP and dFMRP, its *Drosophila* homolog, interact with Dicer, argonaute 1 (AGO1) or AGO2, pre-miRNAs and miRNAs (Caudy et al., 2002; Ishizuka et al., 2002; Caudy and Hannon, 2004; Jin et al., 2004; Plante et al., 2006; Xu et al., 2008; Cheever and Ceman, 2009a,b; Yang et al., 2009; Edbauer et al., 2010; Muddashetty et al., 2011). FMRP has been shown to function with miRNAs and AGO2 to repress the translation of PSD95 and the NMDA receptor subunit NR2A, two important components of the postsynaptic density (Edbauer et al., 2010; Muddashetty et al., 2011). In the case of PSD95, dephosphorylation of FMRP in response to synaptic activity leads to dissociation of FMRP and its target mRNAs from the miRNA-RISC, leading to translational activation (Muddashetty et al., 2011) (Fig. 1). However, the molecules that may function with FMRP in translational activation have yet to be identified.

Together, these results demonstrate an important role for FMRP in controlling activity-dependent local protein synthesis. This suggests that FMRP helps to coordinate synaptic activity with new protein synthesis and synapse remodeling, allowing for certain synapses to be selectively strengthened and others weakened or eliminated.

Synaptic Dysfunction and Altered Cognition in FMRP-Null Organisms

Given that the *Fmr1* gene is highly conserved among species, the strongest evidence for the effect of FMRP on neuronal circuitry and synaptic function has come from model organisms lacking FMRP. By far the most commonly studied model organism has been the *Fmr1* knockout mouse, followed by *dFmr1*-null flies. As mentioned before, in the *Drosophila* genome there is only one *Fmr1* homolog (*dFmr1* or *dFxr*) that shares extensive amino acid sequence identity to the human Fragile X proteins including several key domains (Wan et al., 2000). The protein product of the *dFmr1* gene acts as an RNA-binding protein and can interact with its own mRNA, thus exhibiting a degree of functional conservation (Wan et al., 2000). Thus, *Drosophila* offers a simple model system to study the consequences of loss of FMRP. Importantly, the behavioral outcome of loss-of-function in both flies and mice is consistent with the human phenotypes. The *Fmr1* knockout mouse exhibits increased susceptibility to audiogenic seizures, increased anxiety and locomotor activity, learning and memory deficits and abnormalities with social behavior, phenotypes that recapitulate the increased susceptibility to epileptic seizures, as well as the emotional, behavioral and cognitive deficits seen in human FXS patients (Kooy, 2003; Bear et al., 2004; Hagerman et al., 2009). The *dfmr1*-null fly displays defects in circadian rhythms, sleep, social behaviors and long-term memory (Dockendorff et al., 2002; Inoue et al., 2002; McBride et al., 2005; Bolduc et al., 2008; Bushey et al., 2009). Based on these results in flies, sleep disturbances have recently become a focus in FXS (Kronk et al., 2010). Here, we focus our discussion on the abnormalities in neuronal circuit development, as well as alterations in the structure and function of synapses seen in these two FMRP-null model organisms.

FXS and Dendritic Spine Pathology. A common phenotype seen in several cognitive disorders including FXS is an alteration in the structure and formation of excitatory synapses, in particular, perturbations at postsynaptic sites of glutamate synapses known as dendritic spines. Anatomical studies done in adults suffering from FXS and *Fmr1* knockout mice show abnormalities in dendritic spine properties in the occipital, somatosensory and temporal cortices (Hinton et al., 1991; Comery et al., 1997; Irwin et al., 2000a, 2001; Galvez et al., 2003; Galvez and Greenough, 2005). It is generally accepted that in the normal brain during the first postnatal week dendritic spines are longer than in the adult brain and exhibit a sparse distribution (Fiala et al., 1998; Lendvai et al., 2000). Then by the third week of development spine density increases whereas spine length decreases (Juraska and Fifkova, 1979a,b; Juraska, 1982; Petit et al., 1988). Research done in *Fmr1* knockout mice has revealed that though synaptogenesis and remodeling of synapses does occur in these mice, in the adult organism there remains an abundance of longer and thinner spines, greater spine density, and a greater number of morphologically immature spines in comparison to the adult wild-type counterpart; although the severity of these phenotypes vary considerably based on development and region of the brain (Irwin et al., 2000a,b, 2002; Grossman et al., 2006; Nimchinsky et al., 2001; McKinney et al., 2005). One possible reason for these subtle phenotypes is the idea that FMRP is involved in the experience or activity-dependent refinement of synaptic connections, rather than their initial formation. An

activity-dependent function for FMRP is supported by studies which demonstrate an increase in FMRP in response to experience, environmental enrichment or whisker stimulation (Weiler et al., 1997; Todd and Mack, 2000; Gabel et al., 2004; Irwin et al., 2000b, 2005). More recently, *in vivo* time-lapse imaging using two-photon microscopy has demonstrated a developmental delay in the transition from immature to mature spines and revealed the existence of abnormally unstable spines in the cortex of *Fmr1* knockout mice (Cruz-Martín et al., 2010). In addition, *Fmr1* knockout spines fail to elongate in response to glutamate (Cruz-Martín et al., 2012). These studies suggest that the spatial and temporal expression of FMRP is crucial to the activity-dependent maturation and pruning of synaptic connections that, in turn, is necessary for proper circuit development and cognitive functioning (Bureau et al., 2008; Harlow et al., 2010; Portera-Cailliau, 2012; Xinyuanhe and Portera-Cailliau, 2012).

FMRP, Circuit Remodeling, and Synaptic Plasticity. In support of FMRP's important role in pruning synapses, experiments have shown an increase in functional synapses in cultured *Fmr1* knockout neurons compared with their wild-type neighbors (Pfeiffer and Huber, 2007). In addition, overexpression of FMRP reduces the number of functional and structural synapses in cultured *Fmr1*-null neurons, supporting FMRP's role in synapse elimination (Pfeiffer and Huber, 2007). More recently, Pfeiffer et al. (2010) have demonstrated that FMRP may function alongside the transcription factor myocyte enhancer factor 2 (MEF2) to support activity-dependent synapse elimination (Flavell et al., 2006; Barbosa et al., 2008).

Other key features of neural circuit development include proper outgrowth and pruning of dendrites, as well as proper axonal outgrowth and development of presynaptic terminals. Although FMRP's role in these processes is much less studied, it has been shown to play a role in all of these key aspects of neural circuit development and refinement. For example, *Fmr1* knockout mice display altered dendrite morphologies in the somatosensory cortex and spinal cord (Thomas et al., 2008; Till et al., 2012) and FMRP is required for activity-dependent dendritic remodeling in adult-born granule cells of the mouse olfactory bulb (Scotto-Lomassese et al., 2011). Interestingly, Jacobs et al. (2010a, 2010b) have proposed that glial FMRP may also contribute to dendritic and synaptic maturation. FMRP has also been shown to regulate the expression of several presynaptic proteins and contributes to growth cone motility, growth cone collapse and presynaptic plasticity (Antar et al., 2006; Li et al., 2009; Deng et al., 2011; Klemmer et al., 2011). Similar to the mammalian system, *dFmr1* mutant flies also exhibit defects in synaptic transmission, as well as structural overgrowth and overbranching of pre- and postsynaptic processes both centrally (optic lobes) and peripherally (neuromuscular junction) (Zhang et al., 2001; Gatto and Broadie, 2008; Pan et al., 2004, 2008; Tessier and Broadie, 2008). In both of these areas, it is thought that translation of FMRP target mRNAs fails to be properly repressed, which impacts both the structure and function of synaptic connections. In particular, dFMRP is thought to coordinate activity-dependent dendritic and synaptic remodeling by modulating the translation of the cytoskeleton-associated proteins Futsch (MAP1B) (Zhang et al., 2001), Rac1 GTPase (Schenck et al., 2003), and profilin (Reeve et al., 2005). Therefore, many of FMRP's effects on neural circuit remodeling could be due to an important interaction with mRNAs encoding key components of the actin and microtubule cytoskeleton. This failure to properly prune dendrites and synapses in the absence of FMRP may underlie the hyperconnectivity between certain brain regions that is seen in human FXS patients.

Changes in synaptic activity can lead to long-lasting increases or decreases in synaptic strength. Such changes can be mediated by means of long-term potentiation (LTP; an increase in synapse strength) and long-term depression (LTD; a decrease in synapse strength). In the hippocampus, there are two main types of LTD: one that is dependent on NMDA receptors and one that is dependent on metabotropic glutamate receptors (mGluRs). Though both forms of LTD lead to a decrease in postsynaptic AMPA receptors, their mechanisms of actions are distinct (Carroll et al., 1999; Snyder et al., 2001). The main distinction is that early mGluR-triggered LTD requires the fast translation of mRNAs localized in postsynaptic dendrites, whereas hippocampal NMDA-triggered LTD does not require protein synthesis for its early expression (Huber et al., 2000; Manahan-Vaughan et al., 2000).

Studies have established that FMRP is important for synaptic plasticity, in particular mGluR-dependent LTD. The association between FMRP and mGluR-LTD came from a finding which revealed that synaptoneurosomal activation of Gp1-mGluRs stimulates the synthesis of FMRP (Weiler and Greenough, 1993). Hippocampal slices from *Fmr1* knockout mice were then found to display enhanced, protein synthesis-independent mGluR-LTD, as compared with wild-type slices (Huber et al., 2002; Hou

et al., 2006; Nosyreva and Huber, 2006). Interestingly, this phenotype is specific to mGluR-dependent LTD, as studies have not found a deficit in the early-phase NMDA receptor-dependent LTD (Bear et al., 2004). Based on these results, Bear et al. (2004) put forth the mGluR hypothesis of FXS, which proposes that under normal conditions, increased FMRP synthesis in response to Gp1-mGluR activation maintains the balance of mGluR-LTD by acting as a brake on the synthesis of new proteins upregulated by Gp1-mGluR signaling. Therefore, in the absence of FMRP, Gp1-mGluR dependent mRNA translation continues unopposed, leading to an overabundance of "LTD proteins." Alternatively, global increases in protein synthesis in *Fmr1* knockout mice could lead to exaggerated activation of signaling downstream of Gp1-mGluR receptors (Sharma et al., 2010). By relating these theories to the disease context one can see how the lack of FMRP and the consequential exaggerated activation of Gp1-mGluR signaling could lead to a preponderance of "LTD proteins," synaptic depression and delays in synapse maturation, which may in turn underlie developmental delays and cognitive deficits associated with FXS (Bear et al., 2004; Bhakar et al., 2012).

FMRP has also been shown to be involved in other forms of synaptic plasticity across different brain regions, including certain forms of LTP in the amygdala and cortex (Li et al., 2002; Zhao et al., 2005; Desai et al., 2006; Wilson and Cox, 2007; Suvrathan et al., 2010), LTP priming via mGluR activation in the hippocampus (Auerbach and Bear, 2010) and LTP at the entorhinal cortex-dentate gyrus synapse in the hippocampus (Yun and Trommer, 2011). Changes in E-LTP at the CA3-CA1 synapse in *Fmr1* knockout mice have been observed, but appear to be inconsistent across the literature, perhaps due to differences in FMRP requirement over the course of development (Godfraind et al., 1996; Paradee et al., 1999; Pilpel et al., 2009; Lee et al., 2011). FMRP's role in synaptic plasticity clearly extends beyond the hippocampus and beyond mGluR-LTD, therefore it is of interest to study FMRP's roles in other brain regions, including the amygdala (Suvrathan and Chattarji, 2011) and prefrontal cortex (Krueger et al., 2011), regions thought to underlie many of the symptoms of FXS.

FMRP Homologs and Synaptic Plasticity. Comparatively less is known about phenotypes associated with loss of FXR1P or FXR2P. Interestingly, *Fmr1*/*Fxr2* double knockout mice exhibit a greater enhancement in mGluR-LTD than that seen in the *Fmr1* knockout alone (Zhang et al., 2009). This mGluR-LTD was shown to be only partly dependent on protein synthesis. Late-phase LTP (L-LTP), a form of LTP dependent on rapid new protein synthesis (Kelleher et al., 2004b), remains unperturbed in this double knockout mouse suggesting that both FMRP and FXR2P play a role in mGluR-LTD but neither seems to affect L-LTP (Zhang et al., 2009). Behaviorally, *Fxr2* knockout animals exhibit impairments in context-dependent fear conditioning and perform poorly in the learning phase of the Morris water maze test (Bontekoe et al., 2002). Very little is known about FXR1P since the full mouse knockout of FXR1P dies at birth due to defects in the development of heart and skeletal muscle (Mientjes et al., 2004). Recent evidence has shown that FXR1P directly represses the translation of *desmoplakin*, *talin2*, and its own mRNA in heart muscle, which could potentially explain the lethal phenotype of the full knockout model (Whitman et al., 2011). Further investigation of FXR1P conditional knockout mice is needed to understand the function of this protein in the brain.

FXS and Inhibitory Synapse Function. Although enhanced hippocampal and cortical mGluR-LTD is the most well-characterized deficit in the *Fmr1* knockout mouse (Dölen et al., 2007), a number of studies have also implicated changes in inhibitory synaptic transmission in FXS. As mentioned before, *Fmr1* knockout mice show reduced expression levels of a number of $GABA_A$ receptor subunits, the receptor-type responsible for the majority of the fast inhibitory transmission in the brain (D'Hulst et al., 2006). Consistent with these observations, D'Antuono et al. (2003) have reported a decrease in $GABA_A$ receptor-mediated inhibition in the subiculum (a limbic structure involved in learning and memory) of *Fmr1* knockout brain slices. In addition, dramatic reductions in the frequency and amplitude of phasic IPSCs, tonic inhibitory currents, and in the number of inhibitory synapses are seen in the amygdala of *Fmr1* knockout mice, suggesting fundamental abnormalities in inhibitory fast synaptic and tonic GABAergic transmission (Olmos-Serrano et al., 2010). They also observed an increase in neuronal hyperexcitability in principal neurons of the amygdala which was rescued using the GABA agonist gaboxadol (THIP) (Olmos-Serrano et al., 2010). The amygdala plays an important role in the acquisition and storage of innate and acquired fear memories, as well as in emotional processing within social and nonsocial behavioral contexts (LeDoux, 2003). Amygdala dysfunction is supported in FXS patients as well as in animal behavioral studies showing that

Fmr1 knockouts exhibit abnormal social behavior (McNaughton et al., 2008). Therefore, these studies suggest that both excitatory and inhibitory defects may contribute to the neuronal circuit dysfunction and cognitive abnormalities seen in FXS.

FXS and Intrinsic Excitability. FXS patients display hyperactivity, increased sensitivity to sensory stimuli and a high incidence of epileptic seizures (Gross et al., 2011). Although reduced GABA transmission leading to neuronal hyperexcitability (mentioned above) could help explain some of these phenotypes, more recent studies have found that FMRP also regulates the expression and/or function of several potassium channels, including Slack, Kv3.1b, and Kv4.2, channels known for their role in decreasing neuronal excitability (Brown et al., 2010; Strumbos et al., 2010; Lee et al., 2011). More specifically, brainstem slices from *Fmr1* knockout mice show smaller Slack-mediated currents and display deficits in the function and experience-dependent upregulation of Kv3.1b channels (Brown et al., 2010; Strumbos et al., 2010). FMRP also directly regulates the mRNA translation of the voltage-gated potassium channel Kv4.2, although the exact mechanism by which it does so needs to be clarified (Gross et al., 2011; Lee et al., 2011). Therefore, changes in potassium channel expression and intrinsic excitability may also contribute to the hyperactivity seen in FXS.

FXS and Neurogenesis. Although FMRP's role in controlling synaptic plasticity and experience-dependent neural circuit remodeling has been a major focus of the scientific community thus-far, one cannot forget that FMRP is expressed in a wide variety of cells and tissues during early embryonic development, with gradual restriction to neurons only at later stages of development (Abitbol et al., 1993; Bhakar et al., 2012). This begs the question of whether FMRP is also involved in neurogenesis and/or gliogenesis, the process through which neural stem cells and/or progenitor cells differentiate into neurons and glial cells, respectively. The proper control of neurogenesis and gliogenesis, allowing for the appropriate number of neurons and glial cells to be made at the right time, is critical for the initial stages of neural circuit development (Götz and Huttner, 2005; Callan and Zarnescu, 2011). Indeed, multiple lines of evidence have pointed to a role for FMRP at this crucial developmental check-point. Castrén et al. (2005) demonstrated that neural progenitor cells (NPCs), isolated from *Fmr1* knockout mice or post-mortem human fetuses, formed more neurons and fewer glial cells than wild-type NPCs. The reduction in glial cells was shown to be due to an increase in apoptotic cell death. Furthermore, using bromodeoxyuridine (BrdU) incorporation into replicating DNA, they found an increase in the number of newborn cells in the subventricular zone of *Fmr1* knockout mice (Castrén et al., 2005). In addition, ~30 genes, including receptors, small GTPases and transcription factors, showed altered mRNA levels in Fragile X-NPCs isolated from human fetal cortices, although neurogenesis was unaffected (Bhattacharyya et al., 2008). These results point to a requirement for FMRP in neuron/glia cell fate determination, glial cell survival and early gene expression. Using *Drosophila*, Callan et al. (2010, 2011) extended these findings by demonstrating that dFMRP was required to set the precise timing of re-entry of neural stem cells into the cell cycle. In the absence of dFMRP, neural stem cells exit the quiescent state prematurely, resulting in an overproduction of neurons that persists into adulthood. Loss of FMRP in mice has also been shown to alter cortical plate development by affecting both the transition from radial glial cells to intermediate precursor cells and the differentiation of excitatory glutamatergic cells (Tervonen et al., 2009; Saffary and Xie, 2011), offering further support for the role of FMRP in neurogenesis. Together, these results point to an important, and often overlooked, role for FMRP in the early development of the nervous system (Callan and Zarnescu, 2011).

Interestingly, lack of FMRP from chorionic villi samples of FXS patients is seen only at week 10 of pregnancy, which suggests that early embryogenesis/neurogenesis in both FXS and healthy embryos takes place in the presence of FMRP (Willemsen et al., 2002). Unfortunately, the distinction between FMRP's role during early *versus* late neurogenesis cannot be studied using mouse models, which lack FMRP even at the earliest stages of development. In an attempt to recapitulate the disease process, Telias et al. (2013) isolated Fragile X human embryonic stem cells (FX-hESCs) from male FXS patients carrying the full naturally occurring FXS mutation (>200 CGG repeats). Similar to the disease case, these cells express *Fmr1* in early embryogenesis but show a progressive downregulation of *Fmr1*-expression when induced to differentiate into neurons *in vitro*. This loss of FMRP expression in the early stages of neurogenesis reduced the expression of a number of neural genes known to be important in neurogenesis induction, namely SOX1, NOTCH1, and PAX6, leading to poor neuronal maturation and a larger number of glial cells (Telias et al., 2013). Current and voltage clamp recordings revealed that while FXS-hESC cells can differentiate into viable neurons

with passive electrical properties similar to control cells, they failed to develop functional properties and are unresponsive to glutamate (Telias et al., 2013). Aberrant neuronal differentiation, correlated with loss of FMRP expression, has also been shown using induced pluripotent stem cells isolated from adult FXS patients (Sheridan et al., 2011). These studies offer more evidence in support for FMRP's role in neurogenesis.

An alternative way to probe FMRP's involvement in neurogenesis is to study adult neurogenesis. Adult neurogenesis is a process that persists throughout the entire mammalian lifespan, taking place in the subgranular zone (SGZ) of the dentate gyrus and in the subventricular zone (SVZ) of the lateral ventricles (Ming and Song, 2005). Though the specific purpose of adult neurogenesis is still under debate, evidence shows that the newly synthesized neurons of the dentate gyrus play a critical role in hippocampus-dependent learning due to their lowered thresholds for synaptic plasticity and their ability to integrate in the existing circuitry of the hippocampus (Ming and Song, 2005; Garthe et al., 2009; Deng et al., 2010). *In vivo* and *in vitro* evidence from *Fmr1* knockout mice support a role for FMRP in regulating adult neurogenesis in the mammalian brain (Eadie et al., 2009; Luo et al., 2010; Lazarov et al., 2012). Specifically, loss of FMRP leads to alterations in adult neural progenitor cell (aNPC) proliferation (Luo et al., 2010; Lazarov et al., 2012), decreased survival of aNPCs (Eadie et al., 2009; Lazarov et al., 2012), and changes in cell fate specification (Luo et al., 2010). These effects are proposed to be due to misregulated protein expression/function of several cell cycle and Wnt signaling pathway genes (CDK4, cyclin D1, GSK3β, β-catenin, and neurogenin1), pathways that are known to be involved in stem cell proliferation and differentiation (Luo et al., 2010). Recent evidence reveals that specifically ablating FMRP from aNSCs using inducible gene recombination methods in mice leads to reduced hippocampal adult neurogenesis, as well as impairments in hippocampus-dependent learning (Guo et al., 2011). Unexpectedly, the restoration of FMRP expression specifically in aNSCs was able to rescue hippocampal learning deficits in *Fmr1* knockout mice, suggesting that loss of functional FMRP in aNSCs could be a contributing factor for the learning and memory deficits seen in FXS (Guo et al., 2011). Overall, the research done so far suggests that translational control is an important factor in modulating both embryonic and adult neurogenesis. FMRP may be one of its key players and through this role may be mediating the pathogenesis of intellectual disability seen in the adult brain. In addition, alterations in the numbers of neurons and glial cells may help explain the macroencephaly and changes in the structure and volume of various cortical and subcortical regions in the FXS brain.

Rescue of Phenotypes in the Fmr1 Knockout Mouse. One of the benefits of studying FXS using a bottom-up approach (linking loss of a single gene to disease phenotypes using animal models) has come from studies demonstrating complete or partial rescue of phenotypes using either genetic or pharmacological manipulations (Krueger and Bear, 2011; Bhattacharya et al., 2012; Castrén et al., 2012; Osterweil et al., 2012). Several studies have noted the potential for improvements even in adult mice, long after the critical period thought to be essential for the laying down of behaviors (Krueger and Bear, 2011; Castrén et al., 2012; Michalon et al., 2012). This discovery has opened up the possibility that several human FXS symptoms can be reversed even into adulthood (Castrén et al., 2012; Osterweil et al., 2012).

Strong support for the important role of exaggerated Gp1-mGluR signaling in FXS came from several studies in both mice and *Drosophila* showing rescue of FXS phenotypes following reduced mGluR signaling. Yan et al. (2005) were the first to demonstrate that several behavioral phenotypes in the *Fmr1* knockout mouse are rescued by decreasing mGluR signaling using 2-Methyl-6-(phenylethynyl)pyridine (MPEP), an mGluR5 antagonist. Later, using a genetic approach to reduce mGluR5 expression, investigators were able to prevent the increased spine density, basal protein synthesis, responsiveness to audiogenic seizure and exaggerated inhibitory avoidance extinction seen in the *Fmr1* knockout mice (Dölen et al., 2007). More recently, Michalon et al. (2012) showed that many of the FXS phenotypes could be reversed in adult mice using either acute or chronic treatment with the selective mGluR5 antagonist, CTEP. In addition, the *Drosophila* genome also encodes a single functional mGluR (DmGluRA) which is localized in both synaptic neuropil of the central nervous system and at the neuromuscular junction (Parmentier et al., 1996; Bogdanik et al., 2004). Remarkably, blocking mGluR signaling rescues synaptic and behavioral defects seen in *dFmr1*-null neurons (McBride et al., 2005; Pan et al., 2008). This rescue data further implicates unbalanced mGluR signaling in causing FXS-related phenotypes. Together, these studies have paved the way for several clinical trials using compounds targeting mGluR signaling (Osterweil et al., 2012).

In parallel, researchers have been on the hunt for potential "LTD proteins"— those proteins expressed

downstream of mGluR signaling and found overexpressed in the *Fmr1* knockout mouse—in an attempt to identify other therapeutic targets for FXS. These proteins include the amyloid precursor protein (APP), the striatal-enriched protein tyrosine phosphatase (STEP), metalloproteinase 9 (MMP-9), and glycogen synthase kinase 3 (GSK3). These proteins are synthesized/activated in response to mGluR5 activation and are found at higher than normal expression or activity levels in the *Fmr1* knockout mouse (Westmark and Malter, 2007; Bilousova et al., 2009; Min et al., 2009; Goebel-Goody and Lombroso, 2012). Intriguingly, researchers have found that reducing the expression and/or function of each of these proteins individually, using either genetic or pharmacological approaches, can reverse several FXS phenotypes seen in mice (Bilousova et al., 2009; Mines et al., 2010; Yuskaitis et al., 2010; Siller and Broadie, 2011; Westmark et al., 2011; Goebel-Goody et al., 2012). These surprising results, where rescue is seen just by targeting a single misregulated protein downstream of mGluR signaling, could be explained if these proteins play essential roles in controlling either the functional or structural changes at synapses that are characteristic of mGluR-LTD. Indeed, both STEP and the cleavage product of APP, β-amyloid, are known to trigger AMPA receptor internalization and LTD (Hsieh et al., 2006; Zhang et al., 2008). MMP9 is a secreted extracellular endopeptidase that is responsible for the elongation and thinning of dendritic spines in response to synaptic activity, a phenotype similar to that seen with mGluR5 signaling (Vanderklish and Edelman, 2002; Michaluk et al., 2011). This suggests that MMP9 may play a major role in controlling spine structure downstream of mGluR signaling (Dziembowska and Wlodarczyk, 2012). In addition, GSK3 is a central metabolic regulatory enzyme involved in gene expression, apoptosis, cellular architecture, neurogenesis, cell migration, and axonogenesis through interaction with a number of signaling pathways (Jope and Johnson, 2004) and could potentially play a key role in mGluR-LTD. Therefore, any of these proteins, key players in signaling pathways downstream of mGluR signaling and with important roles in mGluR-LTD, could be considered a good therapeutic target for FXS intervention.

Researchers have also attempted to rescue the increase in dendritic spine number and abundance of long and immature spines seen in *Fmr1* knockout mice by targeting molecules directly involved in actin cytoskeleton remodeling and dendritic spine morphogenesis. Successful rescue has been demonstrated by targeting two of these proteins, p21 activated kinase (PAK) and Rac1 (Hayashi et al., 2007; Bongmba et al., 2011). In particular, inhibition of PAK activity leads to an opposite profile of cortical spine morphology to that seen in FXS (Hayashi et al., 2004). *In vivo* genetic manipulations that inhibit the catalytic activity of PAK in the forebrain of *Fmr1* knockout mice have been successful in partially rescuing some of the FXS-related abnormalities present at the level of synaptic morphology, synaptic plasticity and behavior (Hayashi et al., 2007). Though the exact interaction of PAK with FMRP is still to be resolved, the data suggests that FMRP and PAK could have antagonizing roles in maintaining spine morphology and synaptic function. In contrast, there is evidence to suggest that Rac1, a member of the Rho-family of GTPases, is a *bona fide* FMRP target. *In vivo* co-immunoprecipitation studies in *Drosophila* show that Rac1 mRNA associates with FMRP-mRNA complexes (Lee et al., 2003). More recent evidence shows that lack of FMRP results in excessive synthesis and hyperactivation of Rac1 suggesting that FMRP may be repressing the synthesis of this protein (Bongmba et al., 2011). In addition, inhibition of Rac1 rescues the exaggerated hippocampal LTD in *Fmr1* knockout mice (Huber et al., 2002; Bongmba et al., 2011). These results suggest that FMRP and Rac1 association is important for establishing and maintaining proper synapse structure as well as function. Moreover, reduction of Rac1 activity in *Fmr1* knockouts may be a good target for rescuing some of the phenotypes seen in FXS. However more research needs to be done to understand the precise mechanism of this association and regulation.

One of the overarching themes from the results presented above is that several phenotypes seen in the *Fmr1* knockout mouse can be rescued by renormalizing protein expression levels, either by targeting the upstream signaling molecules (mGluRs) or by targeting the overexpressed proteins themselves. These findings have lead researchers to examine whether directly targeting the protein synthesis machinery itself could be a plausible therapeutic strategy for FXS. Indeed, several researchers have found that signaling pathways involved in activating the general translation machinery (mTOR/ERK) and their downstream effector, p70 ribosomal kinase 1 (S6K1), are overactive in *Fmr1* knockout mice (Sharma et al., 2010; Hoeffer et al., 2012). Recent evidence shows that removal of one copy of S6K1 through genetic deletion from *Fmr1* knockout mice prevents the enhanced phosphorylation of mTOR and its downstream effectors and rescues many of the structural, electrophysiological and behavioral phenotypes (Bhattacharya et al., 2012). Therefore,

directly targeting the protein synthesis machinery may be a viable and important therapeutic strategy for reversing FXS phenotypes (Bhakar et al., 2012; Zoghbi and Bear, 2012).

Since both genetic and environmental factors contribute to the symptoms of FXS, one additional treatment option for FXS, which is likely to be required in combination with pharmacotherapy, is early behavioral intervention/environmental enrichment (Winarni et al., 2012; Castrén et al., 2012). Evidence from *Fmr1* knockout mice supports this strategy (Restivo et al., 2005; Meredith et al., 2007). Behaviorally, *Fmr1* knockout mice reared in enriched environments show a decrease in anxiety levels, as well as improved habituation and exploratory behavior in comparison to *Fmr1* knockout mice reared in standard cages (Restivo et al., 2005). At the neuronal level, environmental enrichment rescues common neuronal morphological features (thinner, longer, and more immature spines) in the visual cortex of *Fmr1* knockout mice, as well as deficits in synaptic plasticity (increased threshold for spike-timing dependent plasticity) in the prefrontal cortex (Restivo et al., 2005; Meredith et al., 2007). In addition, environmental enrichment increases dendritic branching, increases the number of mature spines and selectively increases the expression of the GluA1 AMPA receptor subunit in both genotypes, the expression level of which is known to be reduced in *Fmr1* knockout mice (Restivo et al., 2005). Interestingly, enrichment did not alter FMRP levels in wild-type animals reared in enriched environments, suggesting that environmental stimulation can activate glutamatergic signaling pathways independent from FMRP-expression. Nonetheless, more research needs to be done to understand these FMRP-independent neural plasticity mechanisms that come into play upon environmental stimulation and how they can be targeted to effectively treat the symptoms of FXS.

Together, these preclinical studies in animal models forge an optimistic path forward for the treatment of FXS (Osterweil et al., 2012). Not only do these studies suggest that early pharmacological and behavioral interventions could help improve certain FXS symptoms, but also that treatments targeted later in development could help reverse already established cognitive deficits and behavioral patterns (Krueger and Bear, 2011; Castrén et al., 2012). Together, these studies point to the general hypothesis that re-establishing proper levels of "plasticity" proteins, either by driving the glutamatergic system more through behavioral environment, or by re-balancing the levels of certain key proteins individually or globally by antagonizing the mGluR signaling pathway and/or general translation machinery, can help improve cognitive function and the quality of life of both FXS patients and their caretakers. As proof of this principle, many of the treatments mentioned above have already shown positive results in human FXS patients (Osterweil et al., 2012; Castrén et al., 2012; Winarni et al., 2012).

Misregulated Synaptic Gene Expression as an Underlying Feature in Cognitive Disorders

A common theme that is emerging from a variety of different lines of evidence is that ASDs, as well as other disorders of cognition and language, result from subtle deviations in the expression levels or function of one or a number of synaptic proteins (Kelleher and Bear, 2008; Auerbach et al., 2011; Darnell, 2011; Peça and Feng, 2012; Zoghbi and Bear, 2012). Since the synapse is composed of an interconnected network of proteins, each one intimately dependent on the function of the others, any disturbance in one or many of these proteins is likely to change its overall structure and function (Sakai et al., 2011). These small deviations can therefore lead to a significant impact on the ability of a synapse to remodel in response to experience and on a more global scale, lead to impairments in the brain's ability to interact and learn from the environment; ultimately manifesting as developmental delays, failure to acquire and apply new skills and social and communication issues.

A small number of families afflicted with ASDs carry rare *de novo* point mutations in synaptic proteins such as Neurexin-1, Neuroligin (-1,-3,-4), PSD95, SAP97, SAPAP2, Shank2 and Shank3, which lead to alterations in the level or function of these synaptic proteins rather than their complete loss (Jamain et al., 2003; Bourgeron, 2009; Peça and Feng, 2012). These proteins are concentrated in the presynaptic terminal or postsynaptic density of excitatory synapses and are involved in synapse formation, activity-dependent maturation and synapse stability. In particular, the neuroligins (postsynaptic) and neurexins (presynaptic) function to adhere and stabilize the pre- and postsynaptic sides of synapses and are important players in setting the balance of excitation to inhibition on a neuron, a crucial component of neuronal network stability (Tabuchi et al., 2007; Krueger et al., 2012). The rest of the affected proteins sit just beneath the postsynaptic membrane and serve as scaffolds bridging together glutamatergic receptors, signaling pathways and the actin cytoskeleton, allowing for the faithful transmission and

maintenance of information at synapses (Peça and Feng, 2012). This means that even small deviations in the levels or functions of any of these synaptic proteins can have far-reaching effects on the ability of the synapse to faithfully transmit and remember information.

In addition, several genetic screens from large populations of affected and control populations have identified hundreds of rare, *de novo* mutations and copy number variations that increase the risk for ASDs, as well as the potential for an additive influence of multiple, commonly found gene variants (See article by Guilmatre, this edition) (Sebat et al., 2007; Abrahams and Geschwind, 2008; Iossifov et al., 2012; Klei et al., 2012; Neale et al., 2012; O'Roak et al., 2011, 2012; Sanders et al., 2012; State and Sestan, 2012). Many of the identified genes and genomic segments encode synaptic proteins (Peça and Feng, 2012; Zoghbi and Bear, 2012). Although in most cases the functional consequences of these mutations have not been systematically determined, it is very likely that even small changes in the amount or function of one or several synaptic proteins contributes to the synaptic dysfunction and cognitive deficits seen in ASD.

The identification of single-gene disorders, where a large percentage of afflicted individuals present with one or more of the symptoms of autism, has greatly increased the scope and speed at which researchers can study the underlying cellular and molecular mechanisms altered in these disorders, leading to potential therapeutic treatments that may also function in ASD. As in the case of FXS, the emerging thread in each of these disorders is that the affected proteins are involved in fine tuning the expression levels and/or function of a large number of proteins, often synaptic proteins, at either the transcriptional or translational level. In fact, most of the proteins function as repressors of gene expression, whose loss leads to an overabundance of synaptic proteins, resulting in an inability to properly remodel or prune neuronal connections in response to experience (Kelleher and Bear, 2008; Zoghbi and Bear, 2012). In the following paragraphs we focus specifically on disorders caused by misregulation of the translational machinery, including tuberous sclerosis complex, PTEN hamartoma tumor syndrome and Neurofibromatosis type 1 (NF1).

Tuberous sclerosis complex is a disorder which accounts for 1 to 4% of ASD cases. It is caused by mutations that result in the loss of one functional copy of either TSC1/hamartin or TSC2/tuberin (Kwiatkowski and Manning, 2005; Kelleher and Bear, 2008). These two proteins form a complex that inhibits mTOR (Tee et al., 2002), which, as mentioned earlier, is a master positive regulator of mRNA translation in cells and at synapses. Therefore, loss of TSC1 or TSC2 is predicted to lead to an increase in the translation of mTOR-dependent transcripts (Bhakar et al., 2012). Reduced function of TSC1 and/or TSC2 leads to deficits in glutamatergic signaling, synaptic plasticity, and cognition (von der Brelie et al., 2006; Ehninger et al., 2008; Bateup et al., 2011; Chévere-Torres et al., 2012a,b). More recently, Auerbach et al. (2011) demonstrated a deficit in hippocampal mGluR-LTD and a paradoxical decrease in basal protein synthesis in the TSC2 heterozygote mouse, as well as a deficit in context-discrimination memory. These phenotypes were rescued by increasing mGluR5 signaling or crossing the mouse with the *Fmr1* null mice, leading to the proposal that TSC1/2 and FMRP function to inhibit the translation of two distinct and competing pools of mRNAs involved in synaptic strengthening and synaptic weakening, respectively (Bhakar et al., 2012; State and Sestan, 2012). In addition, patients with PTEN hamartoma tumor syndrome and NF1 also present with autistic features that are caused by the loss of negative regulation on mTOR signaling (Dasgupta and Gutmann, 2003; Kelleher and Bear, 2008). Loss of PTEN results in heightened mTORC1 activity, neuronal hypertrophy and macroencephaly in mice (Backman et al., 2002; Kwon et al., 2006; Meikle et al., 2008). Loss of NF1, an inhibitor of Ras signaling, leads to an upregulation of ERK and mTOR signaling (Dasgupta and Gutmann, 2003). These results suggest that tightly regulated mTOR-dependent protein synthesis is important for synaptic plasticity and cognition.

Further support for the importance of balanced protein expression for optimal long-lasting synaptic plasticity and cognition has come from mouse models where general translation has been enhanced either through the deletion of a translational repressor or through the overexpression of a translational enhancer. As mentioned previously, the phosphorylation status of eIF2α controls general translation initiation. Genetic reduction of phosphorylated eIF2α or deletion of GCN2 or PKR, kinases which control the phosphorylation status of eIF2α, surprisingly lead to enhanced LTP and improved memory formation (Costa-Mattioli et al., 2005, 2007; Zhu et al., 2011). However, these improvements may be offset by enhanced perseveration and impaired behavioral flexibility, as shown in mice lacking PERK, another eIF2α kinase, or FKBP12, an indirect inhibitor of the mTOR pathway (Hoeffer et al., 2008; Trinh et al., 2012). These phenotypes are reminiscent of the

repetitive behaviors and limited interests commonly seen in autism-spectrum disorders. More recently, researchers have looked at the effect of modulating the expression/activity of eIF4E, the cap-binding protein and crucial player in general translation inhibition, on synaptic plasticity and behavior in mice. Interestingly, the promoter region of eIF4E has been found to be mutated in several patients with autism-spectrum disorders, leading to an upregulation in its expression (Neves-Pereira et al., 2009). Deletion of 4E-BP, a protein which normally represses eIF4E function, or overexpression of eIF4E, both lead to synaptic pathophysiology as well as behaviors characteristic of autism-spectrum disorders, including deficits in social interactions, communication and repetitive behaviors (Gkogkas et al., 2013; Santini et al., 2013). These results are consistent with the idea that ASDs are caused by deviations (too much or too little) in the expression levels of synaptic proteins, leading to alterations in synaptic connectivity and network function (Kelleher and Bear, 2008).

CONCLUSIONS AND OUTLOOK FOR THE FUTURE

The study of FXS and FMRP has offered us an unprecedented look at the link between loss of a single gene and its impact on brain development, cognition and behavior. Here we provided a summary of the research to-date demonstrating that FMRP regulates the stability, transport and/or translation of a large number of brain mRNAs. Therefore, FXS results not only from the loss of FMRP, but also from subtle deviations in the expression of many proteins across the brain. These subtle deviations lead to the improper development of neuronal circuits, an inability to remodel synapses in response to experience, and eventually, over the course of development, dramatic changes in information processing and cognitive abilities in both animal models and human FXS patients. This pattern, where too much or too little protein is detrimental to brain development and cognitive abilities, appears to be conserved in many cases of ASD, suggesting that many treatments developed for FXS may also help alleviate some of the symptoms of ASD.

The majority of studies conducted on the *Fmr1* knockout mouse have focused on the role of FMRP in controlling long-term depression in the hippocampus. This research has made significant contributions to the design of therapeutics to treat FXS symptoms, with the first drugs to arise from these studies currently being tested in humans. However, as in humans, loss of FMRP in mice leads to a diverse range of behavioral and cellular phenotypes, some of which are only now beginning to be explored. This is not really surprising since FMRP is expressed in a wide variety of different brain regions, cell types and subcellular locations and functions in an experience or context-dependent manner. New research is expanding the FMRP field beyond the hippocampus into different regions such as the amygdala and prefrontal cortex (Suvrathan and Chattarji, 2011; Krueger et al., 2011). In addition, new roles for FMRP in neurogenesis, inhibitory synapse formation (Paluszkiewicz et al., 2011), dopamine signaling (Wang et al., 2008b; Paul et al., 2013), and intrinsic excitability are currently being explored. These newer studies serve to emphasize that FMRP's roles are widespread and complex and demonstrate how FXS should be viewed as a disorder of the whole brain, rather than due to dysfunction only in one specific brain region. It is hoped that further research into the molecular and cellular dysfunctions underlying FXS will help to clarify FMRP's myriad roles and pave the way for more treatments for this devastating disorder.

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