

Mini-Review

Fragile X Mental Retardation Protein in Plasticity and Disease

Peter K. Todd and James S. Malter*

Department of Pathology, Medical Scientist and Neuroscience Training Program, University of Wisconsin, Madison, Wisconsin

Fragile X syndrome is the most common cause of mental retardation known to be inherited. The syndrome results from the suppressed expression of a single protein, the fragile X mental retardation protein (FMRP). Understanding the function and regulation of FMRP can, therefore, offer insights into both the pathophysiology of fragile X syndrome and the molecular mechanisms of learning and memory. We provide an overview of current concepts of how FMRP functions in the nervous system, with special emphasis on recent evidence that FMRP has a role in metabotropic glutamate receptor-activated protein translation and synaptic plasticity. © 2002 Wiley-Liss, Inc.

Key words: fragile X mental retardation protein; fragile X syndrome; FMRP

FRAGILE X SYNDROME

Fragile X syndrome (FXS) affects approximately 1:4,000 males and 1:8,000 females worldwide, making it the most common known monogenic cause of mental retardation (Turner et al., 1996; de Vries et al., 1997). The syndrome is characterized clinically by mental retardation, with notable deficits in language and executive functions (de Vries et al., 1998). Behaviorally, patients often have problems with hyperactivity, and about 20% of patients meet DSM IV criteria for autism (Hagerman, 1999). In addition, about 20% of FXS males develop seizures during their lives. FXS is also associated with a specific set of physical manifestations, including distinctive facial dysmorphisms, connective tissue abnormalities, and macroorchidism in postpubertal males (de Vries et al., 1998). Most of these same behavioral and physical features are also seen in female patients, although they tend to have a less severe and more variable presentation (Riddle et al., 1998).

Although neuropathological studies reveal few consistent gross morphological changes (Rudelli et al., 1985; Reyniers et al., 1999), FXS patients have an increased number of long, thin dendritic spines (Rudelli et al., 1985; Hinton et al., 1991; Wisniewski et al., 1991; Irwin et al., 2001). Elongated spines are normally present during development, but in FXS they persist into adulthood despite normal neuronal numbers and density within the neocor-

tex (Rudelli et al., 1985; Hinton et al., 1991; Irwin et al., 2001). The specificity of these findings to FXS, though, is suspect, insofar as similar dendritic changes are seen in nonsyndromic mental retardation (Purpura, 1974). However, a mouse model of the disease (FMR-1 knockout mice; see discussion below) shows similar patterns of dendritic dysmorphology (Comery et al., 1997; Nimchinsky et al., 2001).

FXS normally results from the expansion of a CGG repeat in the 5'-untranslated region (UTR) of the fragile X mental retardation gene FMR1 (Oberle et al., 1991; Vincent et al., 1991; Parrish et al., 1994). In normal individuals, this region contains between 5 and 50 repeats with intervening AGG sequences and is relatively stable. In some cases, though, there is repeat expansion to an intermediate length of between 55 and 200 CGGs. The underlying mechanism is unclear, but it can occur with or without loss of the intervening AGG sequences (Dombrowski et al., 2002). Until recently, it was thought that these intermediate "premutations" carriers were asymptomatic, but there is now evidence for premature ovarian failure in female carriers and a parkinsonian-like neurodegenerative disorder in male carriers despite the absence of typical FXS symptoms (Sherman, 2000; Hagerman et al., 2001). These premutations are highly unstable over successive generations and tend to expand. Once the expansion reaches 200 or greater repeats, the 5'-UTR and surrounding regions of the FMR1 gene become hypermethylated and histone deacetylated, resulting in chromatin condensation. These events prevent FMR1 transcription and lead to the absence of the fragile X mental retardation protein (FMRP; Oberle et al., 1991; Verkerk et al., 1991; Yu et al., 1991; Verheij et al., 1993; for review see Jin and Warren, 2000).

*Correspondence to: James S. Malter, MD, K4/812 Clinical Sciences Center, 600 Highland Drive, Madison, WI 53792.
E-mail: jsalter@facstaff.wisc.edu

Received 23 July 2002; Accepted 29 July 2002

In other trinucleotide repeat disorders, such as myotonic dystrophy, the disease symptoms result not only from the altered expression or function of the affected gene but also from the suppressed production of nearby genes on the same chromosome (Cummings and Zoghbi, 2000). This does not appear to be the case in FXS, for which considerable evidence now implicates the loss of FMRP expression as the sole causative agent in the disorder. For example, a number of FXS patients have normal CGG repeats but a deletion or non-sense mutation within the FMR1 gene itself (Trottier et al., 1994; Lugenbeel et al., 1995). These patients generally have a phenotype that is within the clinical spectrum for the syndrome. There is also a reported patient with a very severe FXS phenotype who had a single mis-sense point mutation of I304N in the coding region of the FMR1 gene (De Boule et al., 1993). Furthermore, as discussed below, the FMR gene product is highly expressed in the FXS target tissues and many aspects of the syndrome are reproduced in FMR-1 knockout mice. Thus, although these findings do not rule out the possibility that other closely linked genes have their expression affected by any methylation-dependent down-regulation of FMR1 expression, it appears that such effects are not critical to the major phenotypic features of the disease.

ANIMAL MODELS OF FXS

A strain of FMR-1 knockout mice on an FVB/129 background was produced in 1994. These mice, which survive into adulthood, show many features that are similar to the human phenotype, including macroorchidism, hyperactivity, and increased susceptibility to audiogenic seizures (Dutch-Belgian Fragile X Consortium, 1994). However, some questions about these findings have arisen because of concerns over a possible background strain effect. Recent studies in other strains, though, have provided a number of consensus findings. First, FMR-1 knockout mice have normal long-term potentiation (LTP) in the hippocampus for at least 4 hr after induction (Godfraind et al., 1996; Paradee et al., 1999), although there is one report of a problem with cortical LTP (Li et al., 2002). This relatively intact synaptic plasticity occurs in the face of decreased synaptic activity and connectivity when hippocampal cells derived from knockout mice are grown in dissociated cultures (Braun and Segal, 2000). Second, FMR-1 knockout mice have an increased susceptibility to audiogenic seizures across different strains and ages (Museumci et al., 2000; Chen and Toth, 2001). In contrast, these mice do not have an increased sensitivity to epileptogenic drugs, such as kainic acid or pentylethylenetetrazole (PTZ; Chen and Toth, 2001). Third, FMR-1 knockout mice do have some consistent learning deficits, including an impaired performance in a fear-conditioning paradigm (Paradee et al., 1999). As in FXS patients, FMR-1 mice also have problems with hypersensitivity, including an increase in prepulse inhibition and auditory startle responses (Chen and Toth, 2001; Nielsen et al., 2002). Fourth, as mentioned previously, FMR knockout mice have dendritic spines that are on average longer and

more numerous than those of wild-type controls (Comery et al., 1997; Nimchinsky et al., 2001). These changes are most marked early in development; green fluorescent protein (GFP)-transfected cortical neurons visualized in vivo show robust differences at 1 week postnatally but few or no significant changes by 4 weeks (Nimchinsky et al., 2001). These changes are not seen when neurons derived from knockout mice are grown in either dissociated or organotypic cultures (Braun and Segal, 2000; Nimchinsky et al., 2001).

In addition to mammalian models of FXS, a *Drosophila* homolog of FMRP, dFXR, has recently been identified (Wan et al., 2000). This protein shows about 60% homology to FMRP and its two autosomal homologues in mammals, FXR1 and FXR2. Flies lacking dFXR show significant alterations in synapse size, axonal and dendritic branching and guidance, and synaptic transmission (Zhang et al., 2001). Furthermore, overexpression of dFXR leads to malformation of wings and eyes and an increase in presynaptic bouton size (Wan et al., 2000; Zhang et al., 2001). dFXR null flies also have defects in circadian rhythms and mating behaviors (Dockendorff et al., 2002; Morales et al., 2002).

FMRP

In recent years, focus within the field has turned to identifying the functions of the FMRP (for additional reviews see Khandjian, 1999; Jin and Warren, 2000). There are 12 different FMR1 mRNAs detectable by reverse transcription-polymerase chain reaction (RT-PCR) secondary to alternative splicing at the 3' end, but Northern blot analysis of brain tissue typically reveals a single 4.4 kb message, with some minor, smaller bands (Ashley et al., 1993a). These mRNAs encode for different isoforms of the protein, ranging in size from 70 to 85 kD, with the most common isoform in the brain containing 614 amino acid residues (see Fig. 1; Ashley et al., 1993a). Both FMR1 mRNA and protein are widely expressed throughout the body in development and adulthood, with the highest expression levels found within the brain and testes (Devys et al., 1993). The subcellular localization of the protein is largely cytoplasmic, with high levels found near the rough endoplasmic reticulum (rER; Feng et al., 1997a). In addition, a small amount (5%) of the protein is found within the nucleus (Feng et al., 1997a). In the brain, FMRP expression appears to be highest in neurons with expression throughout the neocortex. Intense staining is seen within the cell bodies of the hippocampus and granular cell layer of the cerebellum, although this may simply reflect the density of cell bodies in these regions. Importantly, the protein is found at the bases of dendrites and within dendritic spines and has been reported for mature presynaptic boutons in the brain (Feng et al., 1997a; Weiler et al., 1997).

Analysis of the amino acid structure reveals the presence of two hnRNP K homology domains (KH domains) and an RGG box, both of which are RNA binding domains (see Fig. 1; Ashley et al., 1993b; Siomi et al., 1993). In vitro, the full-length protein binds with high

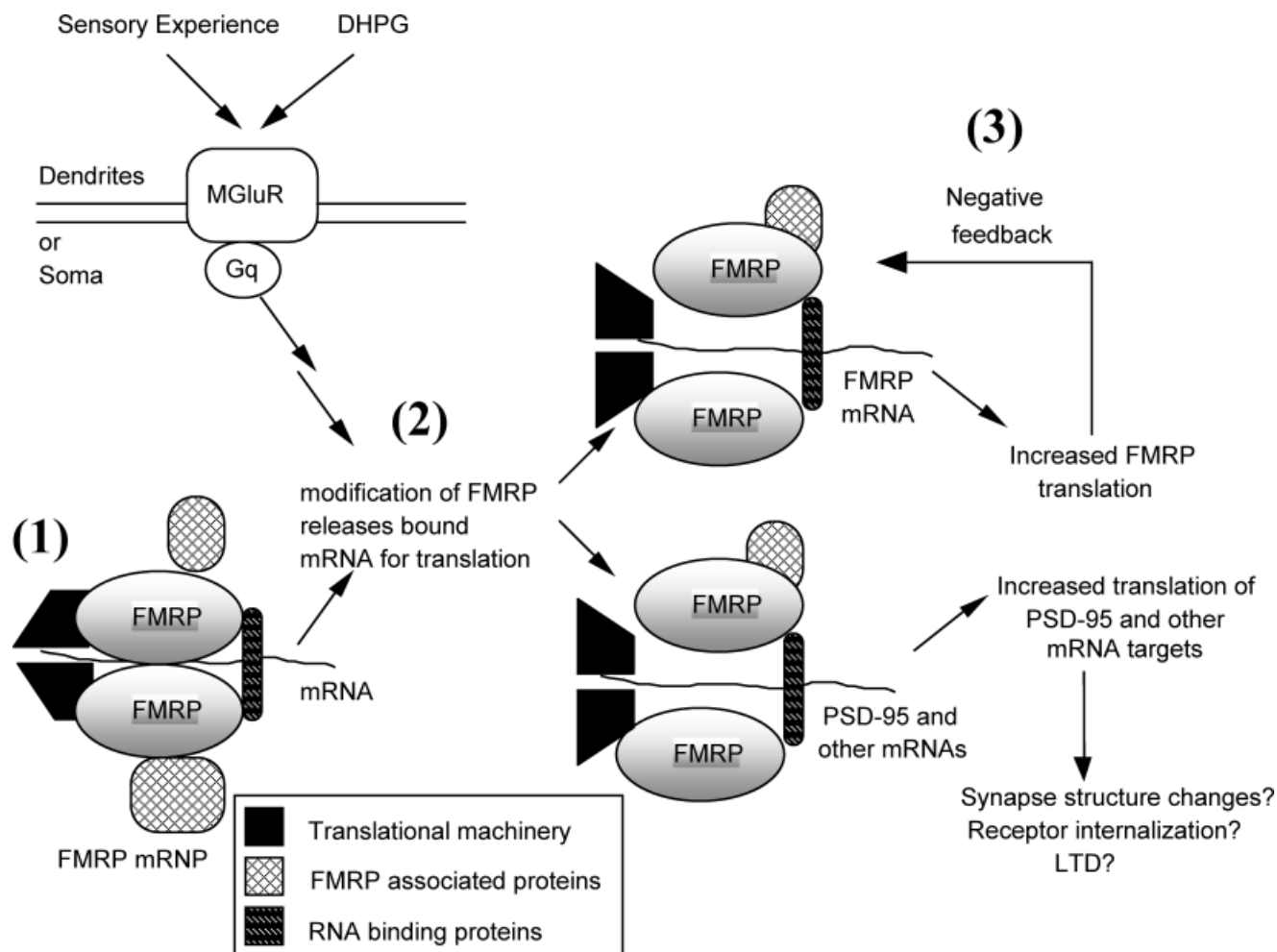


Fig. 1. Hypothetical model of the function of FMRP in the nervous system. Under basal conditions, FMRP is bound to a subset of polysome-associated brain mRNAs and acts to inhibit their translation tonically (1). Stimulation of type I mGluRs by agonists or physiological activation derepresses FMRP's actions on these bound mRNAs, allowing for their rapid translation (2). Among the mRNAs translated is its own mRNA, and this newly produced FMRP can then act to shut off the translational activation by once again binding its target mRNAs (3).

affinity but low specificity to mRNAs, with a preference for poly-G sequences over other single, monoribonucleotide polymers (Ashley et al., 1993b; Siomi et al., 1993; Brown et al., 1998; Lagerbauer et al., 2001). Specific binding of the protein to mRNA was originally thought to be mediated through the two KH domains along with regions within the N-terminus (Siomi et al., 1994; Adinolfi et al., 1999; Sung et al., 2000). Recent work, though, has pointed to the C-terminal region, including the RGG box, as the major site of specific mRNA interaction (Darnell et al., 2001; Schaeffer et al., 2001).

The consensus binding site for FMRP was defined by RNA selection (Selex) and consisted of a set of four quartets of purines that are predicted to form a structure called a *G-quartet* (Darnell et al., 2001). The binding affinity to this quartet by FMRP was near 10 nM and

required the presence of the RGG box but not the KH domains (Darnell et al., 2001). At the same time, another group independently came to similar conclusions through a different approach. Schaeffer and colleagues (2001) defined the sites of interaction between FMRP and the FMR1 mRNA. They found that the highest affinity interactions occurred via the RGG box of FMRP and a G-quartet structure within the 3' coding region of the FMR1 mRNA. Furthermore, when a G-quartet sequence from another gene was swapped with the binding site in FMR1, the interaction was maintained. Taken together, these studies provide strong evidence that G-quartets represent the specific mRNA binding site for FMRP, at least in vitro, and confirm that FMRP binds to its own mRNA (Ashley et al., 1993b).

As well as binding mRNA, FMRP associates with ribosomes both in vivo and in vitro (Khandjian et al., 1996; Feng et al., 1997b). FMRP associates preferentially with polyribosomes, and this association is greatest with actively translating complexes (Corbin et al., 1997; Feng et al., 1997b). The interaction of FMRP with ribosomes is an mRNA-dependent event, which is sensitive both to high salt concentrations and to RNase treatment (Eberhart et al., 1996). However, at least one study suggests that FMRP interacts with the 60S ribosomal subunit via a coiled-coil motif in exon 7 (Siomi et al., 1996). The association with mRNA and the binding of FMRP to polyribosomes are critical to its function in vivo, in that the I304N point mutation that results in a severe clinical phenotype occurs within the second KH domain and disrupts these interactions (Feng et al., 1997b).

Given the ability of FMRP to interact with mRNAs and its association with ribosomes in vivo, it was proposed that FMRP might play a role in regulating the translation of bound mRNAs. There are now some in vitro experiments to support this hypothesis. Recombinant FMRP can inhibit translation with limited specificity in rabbit reticulocyte lysates and injected *Xenopus* oocytes (Laggerbauer et al., 2001; Li et al., 2001). Interestingly, translation inhibition is lost in the I304N point mutant of FMRP, despite normal mRNA binding (Laggerbauer et al., 2001). The inhibition of translation results from a sequestration of the bound mRNAs that prevents ribosomal initiation (Laggerbauer et al., 2001; Li et al., 2001). Although the mechanism of this sequestration is still unclear, there is some evidence that FMRP must first form a homodimer, which the I304N point mutant is unable to do (Laggerbauer et al., 2001). Thus, one function of FMRP may be to inhibit translation of bound mRNAs, but there is still no direct evidence for such inhibition in an in vivo setting.

FMRP-ASSOCIATED PROTEINS AND mRNA LIGANDS

FMRP contains a number of other functional domains, including two coiled-coil domains that mediate protein-protein interactions and both a functional nuclear localization sequence and a nuclear export signal (Eberhart et al., 1996). FMRP interacts with a number of proteins as part of a large, 600 kD messenger ribonucleotide protein complex. These proteins include two autosomal homologs of FMRP, FXR1 and FXR2 (Zhang et al., 1995), the mRNA binding proteins YB-1 and nucleolin (Ceman et al., 1999, 2000), and three novel proteins (NUFIP, CYFIP1, and CYFIP2) that also bind mRNA independently of their interactions with FMRP (Bardoni et al., 1999; Schenck et al., 2001). Furthermore, FMRP can form homomultimers (Tamanini et al., 1999).

Until recently, the only known mRNA binding target for FMRP was its own mRNA (Ashley et al., 1993b). Although initial estimates suggested that up to 4% of total brain mRNA was bound by FMRP, attempts to identify other mRNA targets by FMRP pull-down assays and other in vitro methods have had only limited success (Ashley et al., 1993b; Siomi et al., 1993; Brown et al.,

1998; Sung et al., 2000). Very recently, Brown et al. (2001) immunoprecipitated FMRP from brain lysates of either wild-type or FMR-1 knockout mice and used the associated mRNAs to screen for candidates via cDNA microarrays. The technique yielded more than 100 putative FMRP-associated mRNAs, including numerous molecules that are known to play a role in synaptic plasticity (Brown et al., 2001; Darnell et al., 2001). Furthermore, some of the identified mRNAs contain a G-quartet (Darnell et al., 2001). Although this list is likely neither exhaustive nor completely accurate because of limitations of the technology used, these studies reflect a major step forward in identifying potentially FMRP-responsive mRNAs.

ACTIVITY-DEPENDENT EXPRESSION OF FMRP

There is now considerable evidence both in vitro and in vivo that FMRP expression is an activity-dependent event (Weiler et al., 1997; Irwin et al., 2000; Todd and Mack, 2000). Transgenic mice with a 2.8 kb FMR1 promoter/lac-Z fusion gene, including the FMR1 5'-UTR with the CGG repeat and a CpG island, mimicked normal expression of the gene in vivo (Hergersberg et al., 1995). In vivo footprinting analysis revealed four interreaction sites in the proximal 1.1 kb of the FMR1 promoter that match consensus sequences for NRF1, Sp1, USF1, USF2, and c-myc. Among these, the NRF1 and USF1/2 interactions are the most critical to transcriptional regulation in PC-12 cells (Kumari and Usdin, 2001). Interestingly, binding is methylation sensitive, suggesting that one component of the transcriptional suppression seen in FXS is due to decreased transcription factor binding (Schwemmle et al., 1997; Kumari and Usdin, 2001).

The CGG repeat sequence likely participates in suppressing transcription of FMR-1 under normal conditions through a recently characterized CGG repeat binding protein (CGGBP1, or p20; Muller-Hartmann et al., 2000). Unexpectedly, premutation carriers with expanded repeats actually have a higher level of FMR1 mRNA transcription, but increased FMR1 mRNA is offset by a CGG-mediated translational suppression (Feng et al., 1995; Tassone et al., 2000, 2001; Kenneson et al., 2001). In fact, this CGG-mediated translational suppression is likely the causative agent for FXS in some patients with greater than 200 repeats but unmethylated promoters (Feng et al., 1995; Tassone et al., 2001). These findings suggest not only a possible site of activity-dependent regulation of FMR1 but also a potential evolutionary explanation for the presence of the CGG repeat.

At a posttranscriptional level, the FMR1 mRNA contains an extensive and highly conserved 3'-UTR (>2 kb) and a functional internal ribosomal entry site (IRES) in its 5'-UTR that may be important for translational regulation (Chiang et al., 2001). The first evidence of such regulation came from cultured mouse kidney cells approaching quiescence, in which FMRP levels increased as both the cellular proliferation rate and the FMR1 mRNA levels declined (Khandjian et al., 1995). Further evidence

comes from studies of protein synthesis in synaptoneuro-somes, a subcellular fraction of cortical homogenates enriched for intact pre- and postsynaptic components (Weiler and Greenough, 1993). Application of high potassium or metabotropic glutamate receptor (mGluR) agonists leads to an increase in overall protein synthesis that includes FMRP (Weiler and Greenough, 1993; Weiler et al., 1997).

To date, there are limited data on whether either FMR1 or FMRP levels change in response to neuronal activity *in vivo*. Todd and Mack (2000) showed that FMRP increases in the barrel cortex after whisker stimulation, a model of experience-dependent plasticity, but not after a kainic acid-induced seizure. These changes were seen only in subcellular fractions enriched for synaptosomes and polyribosomes, suggesting site-specific production of the protein. FMRP levels increased in the visual and motor cortices after extended exposure to an enriched environment, although similar changes were also seen after exercise alone, suggesting that the effects may be nonspecific to the environment. In contrast to the case for FMRP, no significant changes in cortical levels of FMR1 mRNA were seen within 2.5 hr after a PTZ-induced seizure (Godfraind et al., 1996) or within 6 hr of induction of *in vivo* hippocampal LTP (Valentine et al., 2000).

FMRP IN mGluR-MEDIATED PLASTICITY

A growing body of evidence now suggests that FMRP plays a role in type I mGluR-mediated plasticity. mGluRs are a large family of G-protein-coupled receptors expressed throughout the nervous system. The two type I mGluRs, mGluR₁ and mGluR₅, are coupled to G_q proteins that activate protein kinase C and release of calcium from intracellular stores via phospholipase C, inositol triphosphate (IP-3), and diacylglycerol (DAG). In addition, activation of type I mGluRs increases conductance through potassium channels and leads to activation of the mitogen-activated protein (MAP) kinase/extracellular signal-related kinase (ERK) pathway through a non-G-coupled mechanism (Bordi and Ugolini, 1999; Ferraguti et al., 1999).

Application of the type I mGluR-specific agonist DHPG induces a dendritic protein synthesis-dependent form of long-term depression (LTD) both in the CA1 region of the hippocampus (Huber et al., 2000) and in the cerebellum (Karachot et al., 2001). DHPG also triggers a protein synthesis-dependent augmentation of epileptiform activity in hippocampal slices and can elicit limbic system seizures *in vivo* (Tizzano et al., 1995; Merlin and Wong, 1997; Merlin et al., 1998). Furthermore, type I mGluR agonists lower the threshold for N-methyl-D-aspartate (NMDA) receptor-mediated LTP in a protein synthesis-dependent fashion (Cohen et al., 1998; Raymond et al., 2000). DHPG application is also associated with internalization of both NMDA and AMPA receptors in dissociated hippocampal cultures (Snyder et al., 2001), and this process is required for DHPG-dependent LTD in the hippocampus (Xiao et al., 2001). Type I mGluRs also play a role in neuronal plasticity and pathology *in vivo*,

insofar as mice lacking either mGluR₁ or mGluR₅ show significant impairments in both the Morris water-maze and fear-conditioning paradigms (Conquet et al., 1994; Lu et al., 1997). Thus, type I mGluR activation is critical for normal cortical development and some forms of synaptic plasticity, and these effects appear to be mediated by a rapid burst of protein synthesis that occurs within 10–15 min of receptor activation (Raymond et al., 2000; Karachot et al., 2001).

Multiple lines of evidence now point to a role for FMRP in these mGluR-mediated processes. First, FMRP inhibits translation of bound mRNAs *in vitro*, including its own mRNA (Laggerbauer et al., 2001; Li et al., 2001), suggesting that FMRP could act as a regulator of activity-dependent translation. Second, FMRP is translated in synaptoneuro-somes in response to activation of type I mGluRs (Weiler et al., 1997). Furthermore, recent unpublished results suggest that mGluR activation of protein synthesis is reduced in synaptoneuro-somes derived from FMR-1 knockout mice, implicating FMRP as a necessary component in mGluR-dependent translational activation (Greenough et al., 2001). Finally, mGluR-dependent LTD in the hippocampus has recently been shown to be enhanced in slices from FMR-1 knockout mice (Huber et al., 2002).

Data from our laboratory add further support to the hypothesis that FMRP is involved in mGluR-mediated plasticity. First, we find that whisker stimulation-dependent increases in FMRP involve type I mGluR activation and require new protein translation but that they are not associated with any changes in FMR1 mRNA production, suggesting posttranscriptional and mGluR-dependent regulation *in vivo* (Todd et al., 2002b). Second, *in vitro* stimulation of primary cortical neurons with the type I mGluR agonist DHPG rapidly increases translation of FMRP (Todd et al., 2002a). Concomitantly, PSD-95 is also rapidly translated in response to mGluR activation, with maximal responses by 20 min from the onset of stimulation in both the dendrites and the cell soma. Of note is that these mGluR-dependent increases in PSD-95 expression are absent in neurons derived from FMR-1 knockout mice (Todd et al., 2002a). Analysis of the 3'-UTR of PSD-95 reveals a near-canonical G-quartet sequence that may serve as a binding site for FMRP.

Therefore, we propose the following working model for the function of FMRP in the nervous system (see Fig. 1). Under basal conditions, FMRP is associated with a subset of G-quartet-containing mRNAs, including PSD-95 and FMRP, in association with numerous other proteins and ribosomes in both the soma and the dendrites. In the absence of activity, FMRP acts as a tonic inhibitor of translation of bound mRNAs. Translational inhibition may require formation of an FMRP homodimer complex and an intact KH domain (Laggerbauer et al., 2001; Ostareck et al., 2001). Activation of type I mGluRs, by the pharmacological agonist DHPG, patterned stimulation (e.g., PP-LFS) in slices, or physiological experience *in vivo*, leads to the FMRP modification, which derepresses

the translational inhibition. Because these mRNAs are already associated with the translational machinery (Corbin et al., 1997; Feng et al., 1997b), they are immediately and reiteratively translated, leading to a rapid and robust site-specific increase in FMRP, PSD-95, and protein products of other FMRP target mRNAs. The increased PSD-95 and other FMRP target proteins may act as “effector” molecules that mediate the protein synthesis-dependent forms of mGluR plasticity, such as LTD, LTP priming, and augmentation of epileptiform activity. This increase in protein translation is transient; both FMRP and PSD-95 reach maximal levels by 20 min, and mGluR-dependent forms of plasticity, such as cerebellar LTD, require protein synthesis for only 15 min (Raymond et al., 2000; Karachot et al., 2001). Thus, this “pulse” of new protein synthesis must be rapidly repressed, possibly as a result of translational inhibition by newly produced (and unmodified) FMRP (see Fig. 1). In agreement with this hypothesis, addition of recombinant (and previously unmodified) FMRP to a rabbit reticulocyte lysate suppresses translation despite significant amounts of FMRP already present (Li et al., 2001).

For FXS, we predict a loss of temporally and site-specific mGluR-dependent translation of FMRP-bound mRNAs. As is the case for PSD-95, there may be no change in the basal expression of these proteins because of translation in a non-FMRP-dependent fashion. Despite the presence of the two FMRP homologues, FXR1 and FXR2, which may partially compensate for the absence of FMRP, mGluR-coupled translation remains deficient. This is consistent with data showing that neither FXR1 nor FXR2 can inhibit the translation of mRNAs *in vitro* (Laggerbauer et al., 2001).

CONCLUSIONS AND FUTURE DIRECTIONS

FXS represents both a significant cause of mental retardation and a model system for the study of learning and memory. The syndrome results from the lack of expression of a single protein, FMRP, which acts as a regulator of translation *in vitro* and is associated with mRNAs and ribosomes *in vivo*. It appears, based on the available evidence, that FMRP may play a role in the transport, localization, and activity-dependent translation of bound mRNAs in the nervous system. Future studies will be needed to delineate both the mechanisms by which FMRP acts to regulate the translation of bound mRNAs and how these newly synthesized proteins influence synaptic plasticity. Still, we are approaching a mechanistic understanding of the molecular systems that are affected in FXS, and for the first time we can speak with optimism about the possibilities of developing rational and effective therapies for patients.

REFERENCES

- Adinolfi S, Bagni C, Musco G, Gibson T, Mazzarella L, Pastore A. 1999. Dissecting FMR1, the protein responsible for fragile X syndrome, in its structural and functional domains. *RNA* 5:1248–1258.
- Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, Warren ST. 1993a. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. *Nat Genet* 4:244–251.
- Ashley CT Jr, Wilkinson KD, Reines D, Warren ST. 1993b. FMR1 protein: conserved RNP family domains and selective RNA binding. *Science* 262:563–566.
- Bardoni B, Schenck A, Mandel JL. 1999. A novel RNA-binding nuclear protein that interacts with the fragile X mental retardation (FMR1) protein. *Hum Mol Genet* 8:2557–2566.
- Bordi F, Ugolini A. 1999. Group I metabotropic glutamate receptors: implications for brain diseases. *Progr Neurobiol* 59:55–79.
- Braun K, Segal M. 2000. FMRP involvement in formation of synapses among cultured hippocampal neurons. *Cereb Cortex* 10:1045–1052.
- Brown V, Small K, Lakkis L, Feng Y, Gunter C, Wilkinson KD, Warren ST. 1998. Purified recombinant FMRP exhibits selective RNA binding as an intrinsic property of the fragile X mental retardation protein. *J Biol Chem* 273:15521–15527.
- Brown V, Jin P, Ceman S, Darnell JC, O'Donnell WT, Tenenbaum SA, Jin X, Feng Y, Wilkinson KD, Keene JD, Darnell RB, Warren ST. 2001. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell* 107:477–487.
- Ceman S, Brown V, Warren ST. 1999. Isolation of an FMRP-associated messenger ribonucleoprotein particle and identification of nucleolin and the fragile X-related proteins as components of the complex. *Mol Cell Biol* 19:7925–7932.
- Ceman S, Nelson R, Warren ST. 2000. Identification of mouse YB1/p50 as a component of the FMRP-associated mRNP particle. *Biochem Biophys Res Commun* 279:904–908.
- Chen L, Toth M. 2001. Fragile X mice develop sensory hyperreactivity to auditory stimuli. *Neuroscience* 103:1043–1050.
- Chiang PW, Carpenter LE, Hagerman PJ. 2001. The 5'-untranslated region of the FMR1 message facilitates translation by internal ribosome entry. *J Biol Chem* 276:37916–37921.
- Cohen AS, Raymond CR, Abraham WC. 1998. Priming of long-term potentiation induced by activation of metabotropic glutamate receptors coupled to phospholipase C. *Hippocampus* 8:160–170.
- Comery TA, Harris JB, Willems PJ, Oostra BA, Irwin SA, Weiler IJ, Greenough WT. 1997. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc Natl Acad Sci USA* 94:5401–5404.
- Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, Bordi F, Franz-Bacon K, Reggiani A, Matarese V, Conde F. 1994. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature* 372:237–243.
- Corbin F, Bouillon M, Fortin A, Morin S, Rousseau F, Khandjian EW. 1997. The fragile X mental retardation protein is associated with poly(A)⁺ mRNA in actively translating polyribosomes. *Hum Mol Genet* 6:1465–1472.
- Cummings CJ, Zoghbi HY. 2000. Trinucleotide repeats: mechanisms and pathophysiology. *Annu Rev Genomics Hum Genet* 1:281–328.
- Darnell JC, Jensen KB, Jin P, Brown V, Warren ST, Darnell RB. 2001. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 107:489–499.
- De Boule K, Verkerk AJ, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van den Bos F, de Graaff E, Oostra BA, Willems PJ. 1993. A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat Genet* 3:31–35.
- de Vries BB, van den Ouweland AM, Mohkamsing S, Duivenvoorden HJ, Mol E, Gelsema K, van Rijn M, Halley DJ, Sandkuijl LA, Oostra BA, Tibben A, Niermeijer MF. 1997. Screening and diagnosis for the fragile X syndrome among the mentally retarded: an epidemiological and psychological survey. Collaborative Fragile X Study Group. *Am J Hum Genet* 61:660–667.

- de Vries BB, Halley DJ, Oostra BA, Niermeijer MF. 1998. The fragile X syndrome. *J Med Genet* 35:579–589.
- Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL. 1993. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat Genet* 4:335–340.
- Dockendorff TC, Su HS, McBride SM, Yang Z, Choi CH, Siwicki KK, Sehgal A, Jongens TA. 2002. *Drosophila* lacking *dfmr1* activity show defects in circadian output and fail to maintain courtship interest. *Neuron* 34:973–984.
- Dombrowski C, Levesque S, Morel ML, Rouillard P, Morgan K, Rousseau F. 2002. Premutation and intermediate-size FMR1 alleles in 10,572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. *Hum Mol Genet* 11:371–378.
- Dutch-Belgian Fragile X Consortium. 1994. *Fmr1* knockout mice: a model to study fragile X mental retardation. *Cell* 78:23–33.
- Eberhart DE, Malter HE, Feng Y, Warren ST. 1996. The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. *Hum Mol Genet* 5:1083–1091.
- Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, Warren ST. 1995. Translational suppression by trinucleotide repeat expansion at FMR1. *Science* 268:731–734.
- Feng Y, Gutekunst CA, Eberhart DE, Yi H, Warren ST, Hersch SM. 1997a. Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. *J Neurosci* 17:1539–1547.
- Feng Y, Absher D, Eberhart DE, Brown V, Malter HE, Warren ST. 1997b. FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. *Mol Cell* 1:109–118.
- Ferraguti F, Baldani-Guerra B, Corsi M, Nakanishi S, Corti C. 1999. Activation of the extracellular signal-regulated kinase 2 by metabotropic glutamate receptors. *Eur J Neurosci* 11:2073–2082.
- Godfraind JM, Reyniers E, De Boule K, D'Hooge R, De Deyn PP, Bakker CE, Oostra BA, Kooy RF, Willems PJ. 1996. Long-term potentiation in the hippocampus of fragile X knockout mice. *Am J Med Genet* 64:246–251.
- Greenough WT, Klintsova AY, Irwin SA, Galvez R, Bates KE, Weiler JJ. 2001. Synaptic regulation of protein synthesis and the fragile X protein. *Proc Natl Acad Sci USA* 98:7101–7106.
- Hagerman RJ. 1999. Fragile X syndrome. In: Hagerman RJ, editor. *Neurodevelopmental disorders: diagnosis and treatment*. New York: Oxford University Press. p 61–132.
- Hagerman RJ, Leehy M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ. 2001. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology* 57:127–130.
- Hergersberg M, Matsuo K, Gassmann M, Schaffner W, Luscher B, Rulicke T, Aguzzi A. 1995. Tissue-specific expression of a FMR1/beta-galactosidase fusion gene in transgenic mice. *Hum Mol Genet* 4:359–366.
- Hinton VJ, Brown WT, Wisniewski K, Rudelli RD. 1991. Analysis of neocortex in three males with the fragile X syndrome. *Am J Med Genet* 41:289–294.
- Huber KM, Kayser MS, Bear MF. 2000. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* 288:1254–1257.
- Huber KM, Gallagher SM, Warren ST, Bear M. 2002. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci USA* 99:7746–7750.
- Irwin SA, Swain RA, Christmon CA, Chakravarti A, Weiler JJ, Greenough WT. 2000. Evidence for altered fragile-X mental retardation protein expression in response to behavioral stimulation. *Neurobiol Learn Mem* 74:87–93.
- Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy F, Willems PJ, Cras P, Kozlowski PB, Swain RA, Weiler JJ, Greenough WT. 2001. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am J Med Genet* 98:161–167.
- Jin P, Warren ST. 2000. Understanding the molecular basis of fragile X syndrome. *Hum Mol Genet* 9:901–908.
- Karachot L, Shirai Y, Vigot R, Yamamori T, Ito M. 2001. Induction of long-term depression in cerebellar Purkinje cells requires a rapidly turned over protein. *J Neurophysiol* 86:280–289.
- Kenneson A, Zhang F, Hagedorn CH, Warren ST. 2001. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet* 10:1449–1454.
- Khandjian EW. 1999. Biology of the fragile X mental retardation protein, an RNA-binding protein. *Biochem Cell Biol* 77:331–342.
- Khandjian EW, Fortin A, Thibodeau A, Tremblay S, Cote F, Devys D, Mandel JL, Rousseau F. 1995. A heterogeneous set of FMR1 proteins is widely distributed in mouse tissues and is modulated in cell culture. *Hum Mol Genet* 4:783–789.
- Khandjian EW, Corbin F, Woerly S, Rousseau F. 1996. The fragile X mental retardation protein is associated with ribosomes. *Nat Genet* 12:91–93.
- Kumari D, Usdin K. 2001. Interaction of the transcription factors USF1, USF2, and alpha-Pal/Nrf-1 with the FMR1 promoter. Implications for fragile X mental retardation syndrome. *J Biol Chem* 276:4357–4364.
- Laggerbauer B, Ostareck D, Keidel EM, Ostareck-Lederer A, Fischer U. 2001. Evidence that fragile X mental retardation protein is a negative regulator of translation. *Hum Mol Genet* 10:329–338.
- Li J, Pelletier MR, Perez Velazquez JL, Carlen PL. 2002. Reduced cortical synaptic plasticity and GluR1 expression associated with fragile X mental retardation protein deficiency. *Mol Cell Neurosci* 19:138–151.
- Li Z, Zhang Y, Ku L, Wilkinson KD, Warren ST, Feng Y. 2001. The fragile X mental retardation protein inhibits translation via interacting with mRNA. *Nucleic Acids Res* 29:2276–2283.
- Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC. 1997. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci* 17:5196–5205.
- Lugenbeel KA, Peier AM, Carson NL, Chudley AE, Nelson DL. 1995. Intragenic loss of function mutations demonstrate the primary role of FMR1 in fragile X syndrome. *Nat Genet* 10:483–485.
- Merlin LR, Wong RK. 1997. Role of group I metabotropic glutamate receptors in the patterning of epileptiform activities in vitro. *J Neurophysiol* 78:539–544.
- Merlin LR, Bergold PJ, Wong RK. 1998. Requirement of protein synthesis for group I mGluR-mediated induction of epileptiform discharges. *J Neurophysiol* 80:989–993.
- Morales J, Hiesinger PR, Schroeder AJ, Kume K, Verstreken P, Jackson FR, Nelson DL, Hassan BA. 2002. *Drosophila* fragile X protein, DFXR, regulates neuronal morphology and function in the brain. *Neuron* 34:961–972.
- Muller-Hartmann H, Deissler H, Naumann F, Schmitz B, Schroer J, Doerfler W. 2000. The human 20-kDa 5'-(CGG)(n)-3'-binding protein is targeted to the nucleus and affects the activity of the FMR1 promoter. *J Biol Chem* 275:6447–6452.
- Musumeci SA, Bosco P, Calabrese G, Bakker C, De Sarro GB, Elia M, Ferri R, Oostra BA. 2000. Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. *Epilepsia* 41:19–23.
- Nielsen DM, Derber WJ, McClellan DA, Crnic LS. 2002. Alterations in the auditory startle response in *Fmr1* targeted mutant mouse models of fragile X syndrome. *Brain Res* 927:8–17.
- Nimchinsky EA, Oberlander AM, Svoboda K. 2001. Abnormal development of dendritic spines in FMR1 knock-out mice. *J Neurosci* 21:5139–5146.

- Oberle I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, Bertheas MF, Mandel JL. 1991. Instability of a 550-base-pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 252:1097–1102.
- Ostareck DH, Ostareck-Lederer A, Shatsky IN, Hentze MW. 2001. Lipoygenase mRNA silencing in erythroid differentiation: the 3'-UTR regulatory complex controls 60S ribosomal subunit joining. *Cell* 104:281–290.
- Paradee W, Melikian HE, Rasmussen DL, Kenneson A, Conn PJ, Warren ST. 1999. Fragile X mouse: strain effects of knockout phenotype and evidence suggesting deficient amygdala function. *Neuroscience* 94:185–192.
- Parrish JE, Oostra BA, Verkerk AJ, Richards CS, Reynolds J, Spikes AS, Shaffer LG, Nelson DL. 1994. Isolation of a GCC repeat showing expansion in FRAXF, a fragile site distal to FRAXA and FRAXE. *Nat Genet* 8:229–235.
- Purpura DP. 1974. Dendritic spine “dysgenesis” and mental retardation. *Science* 186:1126–1128.
- Raymond CR, Thompson VL, Tate WP, Abraham WC. 2000. Metabotropic glutamate receptors trigger homosynaptic protein synthesis to prolong long-term potentiation. *J Neurosci* 20:969–976.
- Reyniers E, Martin JJ, Cras P, Van Marck E, Handig I, Jorens HZ, Oostra BA, Kooy RF, Willems PJ. 1999. Postmortem examination of two fragile X brothers with an FMR1 full mutation. *Am J Med Genet* 84:245–249.
- Riddle JE, Cheema A, Sobesky WE, Gardner SC, Taylor AK, Pennington BF, Hagerman RJ. 1998. Phenotypic involvement in females with the FMR1 gene mutation. *Am J Ment Retard* 102:590–601.
- Rudelli RD, Brown WT, Wisniewski K, Jenkins EC, Laure-Kamionowska M, Connell F, Wisniewski HM. 1985. Adult fragile X syndrome. Cliniconeuropathologic findings. *Acta Neuropathol* 67:289–295.
- Schaeffer C, Bardoni B, Mandel JL, Ehresmann B, Ehresmann C, Moine H. 2001. The fragile X mental retardation protein binds specifically to its mRNA via a purine quartet motif. *EMBO J* 20:4803–4813.
- Schenck A, Bardoni B, Moro A, Bagni C, Mandel JL. 2001. A highly conserved protein family interacting with the fragile X mental retardation protein (FMRP) and displaying selective interactions with FMRP-related proteins FXR1P and FXR2P. *Proc Natl Acad Sci USA* 98:8844–8849.
- Schwemmler S, de Graaff E, Deissler H, Glaser D, Wohlr D, Kennerknecht I, Just W, Oostra BA, Dorfler W, Vogel W, Steinbach P. 1997. Characterization of FMR1 promoter elements by in vivo-footprinting analysis. *Am J Hum Genet* 60:1354–1362.
- Sherman SL. 2000. Premature ovarian failure in the fragile X syndrome. *Am J Med Genet* 97:189–194.
- Siomi H, Siomi MC, Nussbaum RL, Dreyfuss G. 1993. The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. *Cell* 74:291–298.
- Siomi H, Choi M, Siomi MC, Nussbaum RL, Dreyfuss G. 1994. Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell* 77:33–39.
- Siomi MC, Zhang Y, Siomi H, Dreyfuss G. 1996. Specific sequences in the fragile X syndrome protein FMR1 and the FXR proteins mediate their binding to 60S ribosomal subunits and the interactions among them. *Mol Cell Biol* 16:3825–3832.
- Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF. 2001. Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat Neurosci* 4:1079–1085.
- Sung YJ, Conti J, Currie JR, Brown WT, Denman RB. 2000. RNAs that interact with the fragile X syndrome RNA binding protein FMRP. *Biochem Biophys Res Commun* 275:973–980.
- Tamanini F, Van Unen L, Bakker C, Sacchi N, Galjaard H, Oostra BA, Hoogeveen AT. 1999. Oligomerization properties of fragile-X mental-retardation protein (FMRP) and the fragile-X-related proteins FXR1P and FXR2P. *Biochem J* 343(Pt 3):517–523.
- Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. 2000. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am J Hum Genet* 66:6–15.
- Tassone F, Hagerman RJ, Taylor AK, Hagerman PJ. 2001. A majority of fragile X males with methylated, full mutation alleles have significant levels of FMR1 messenger RNA. *J Med Genet* 38:453–456.
- Tizzano JP, Griffey KI, Schoepp DD. 1995. Induction or protection of limbic seizures in mice by mGluR subtype selective agonists. *Neuropharmacology* 34:1063–1067.
- Todd PK, Mack KJ. 2000. Sensory stimulation increases cortical expression of the fragile X mental retardation protein in vivo. *Brain Res Mol Brain Res* 80:17–25.
- Todd PK, Mack KJ, Malter JS. 2002a. The fragile X mental retardation protein is required for mGluR dependent translation of PSD-95. Submitted for publication.
- Todd PK, Malter JS, Mack KJ. 2002b. Whisker stimulation-dependent translation of FMRP in the barrel cortex requires activation of type one metabotropic glutamate receptors. *Brain Res Mol Brain Res* (in press).
- Trottier Y, Imbert G, Poustka A, Fryns JP, Mandel JL. 1994. Male with typical fragile X phenotype is deleted for part of the FMR1 gene and for about 100 kb of upstream region. *Am J Med Genet* 51:454–457.
- Turner G, Webb T, Wake S, Robinson H. 1996. Prevalence of fragile X syndrome. *Am J Med Genet* 64:196–197.
- Valentine G, Chakravarty S, Sarvey J, Bramham C, Herkenham M. 2000. Fragile X (*fmr1*) mRNA expression is differentially regulated in two adult models of activity-dependent gene expression. *Brain Res Mol Brain Res* 75:337–341.
- Verheij C, Bakker CE, de Graaff E, Keulemans J, Willemsen R, Verkerk AJ, Galjaard H, Reuser AJ, Hoogeveen AT, Oostra BA. 1993. Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. *Nature* 363:722–724.
- Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP. 1991. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65:905–914.
- Vincent A, Heitz D, Petit C, Kretz C, Oberle I, Mandel JL. 1991. Abnormal pattern detected in fragile-X patients by pulsed-field gel electrophoresis. *Nature* 349:624–626.
- Wan L, Dockendorff TC, Jongens TA, Dreyfuss G. 2000. Characterization of dFMR1, a *Drosophila melanogaster* homolog of the fragile X mental retardation protein. *Mol Cell Biol* 20:8536–8547.
- Weiler IJ, Greenough WT. 1993. Metabotropic glutamate receptors trigger postsynaptic protein synthesis. *Proc Natl Acad Sci USA* 90:7168–7171.
- Weiler IJ, Irwin SA, Klintsova AY, Spencer CM, Brazelton AD, Miyashiro K, Comery TA, Patel B, Eberwine J, Greenough WT. 1997. Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Proc Natl Acad Sci USA* 94:5395–5400.
- Wisniewski KE, Segan SM, Miezieski CM, Sersen EA, Rudelli RD. 1991. The Fra(X) syndrome: neurological, electrophysiological, and neuropathological abnormalities. *Am J Med Genet* 38:476–480.
- Xiao MY, Zhou Q, Nicoll RA. 2001. Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. *Neuropharmacology* 41:664–671.
- Yu S, Pritchard M, Kremer E, Lynch M, Nancarrow J, Baker E, Holman K, Mulley JC, Warren ST, Schlessinger D. 1991. Fragile X genotype characterized by an unstable region of DNA. *Science* 252:1179–1181.
- Zhang Y, O'Connor JP, Siomi MC, Srinivasan S, Dutra A, Nussbaum RL, Dreyfuss G. 1995. The fragile X mental retardation syndrome protein interacts with novel homologs FXR1 and FXR2. *EMBO J* 14:5358–5366.
- Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, Rubin GM, Broadie K. 2001. *Drosophila* fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. *Cell* 107:591–603.