

History Tends to Repeat: *FMR-1* Silencing in Fragile X Syndrome

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Abstract

Fragile X syndrome is the most common form of inherited mental retardation, and afflicts 1 in 1250 males and 1 in 2500 females. The symptoms include connective tissue dysplasia, mental retardation, and macroorchidism (enlarged testis). My lab discovered that the most common forms of this disorder are caused by the expansion of CGG tri-nucleotide repeats on the X chromosome at the FMR-1 gene locus; an excess of 200 repeats in diseased individuals suppresses the translation of FMR-1. The CGG repeat expansion leads to hypermethylation of a CpG island distal to the repeat, leading to transcriptional repression of FMR-1. This gene silencing is also aided by RNA interference and de-acetylation of histones H3 and H4. The FMR-1 gene codes for fragile X mental retardation protein (FMRP), which plays a functional role in protein translation in neurons. FMRP selectively binds to specific mRNAs essential to development of the brain and other parts of the body, and plays a major role in shuttling its ligands from the nucleoplasm to the dendritic cytoplasm. FMRP knockout mice models demonstrate abnormal dendritic spine growth, suggesting altered synaptic plasticity, which may be responsible for the fragile X phenotype. Elucidating the fragile X mechanism of pathogenesis can aid the development of possible treatments to the world's leading cause of mental retardation.

Introduction

Fragile X syndrome is one of the most prevalent forms of mental retardation affecting approximately 1 in 4,000 males and 1 in 8,000 females. FMR1 has been identified as the gene associated with fragile X syndrome (1, 2, 6). This gene was mapped revealing a CGG tri-nucleotide repeat in the 5' non-coding region of the gene. This region was noted to expand in repeat length in fragile X syndrome (1). The expansions of the CGG repeat to levels of 200 repeats or greater is responsible for the instability of the fragile site on the X chromosome. Normal cases possess an average of 30-50 repeats of the CGG region (27). This instability results in the hypermethylation of a CpG island distal to the tri-nucleotide repeat (6,1,26,35). Examination of fragile X patients has consistently shown that the hypermethylation of the CpG island is the primary factor implementing the fragile X phenotype being that methylation is responsible for approximately 99% of known fragile X phenotypes (35, 41). A representation

of the FMR1 gene with binding sites and the CpG island is depicted in Figure 1.

Studies have revealed a significant decrease in the expression of FMR1 mRNA in fragile X cases as a result of the hypermethylation of the CpG island (35). An important feature of repressing of this particular gene is the presence of FMR1 in fetal tissue. The varying levels of expression between normal and fragile X fetal tissue reflects a very important role in development. This data also suggests early acquisition of the methylation, probably during embryogenesis (38,40).

Early acquisition of methylation points out the importance of the loss of FMR1 expression. The human FMR1 gene, being highly conserved among species (16), has been shown to display alternative splicing properties suggesting the presence of many isoforms (4). Mouse models have been developed to study fragile X syndrome due to the allelic similarities (16). Three different levels of repeat within the gene have been reported: normal (7-50 repeats), permutation (50-200 repeats), and full mutation (> 200 repeats). The class of permutation is interesting because it only becomes affective in successive generations when the repeat region has a chance to expand leading to Shermans's paradox. The permutation allele is unstable when transmitted from generation to generation.

All males with the full mutation display the fragile X phenotype where only approximately 50% of females with the mutation display the fragile X phenotype (4, 40). Taken together with data from our more recent studies this proves that the germ line is susceptible to full expansion (32). There seem to be no new mutations appearing to cause fragile X syndrome.

The only known mutation of resulting in fragile X syndrome interestingly shows increases in the transcription of FMR1 have been proportionally linked to the CGG tri-nucleotide repeat length and the repeat number approaches 200 in permutations. As the number of repeats increases the number of FMR1 mRNA levels increase. Unexpectedly, the FMR1 protein products decrease in relation to the CGG repeat length in permutation (25). This finding shows the development of the disease at various points of repeat length during the permutation stage of fragile X syndrome.

Upon extending from the permutation to the full mutation, the FMR1 gene is silenced. The silencing of the FMR1 gene is interesting because it remains unclear as to how the tri-nucleotide repeat expansion occurs in an organism. Moreover, questions still remain as to how the loss of the FMR1 protein, FMRP can have such broad effects as those observed in fragile X syndrome (21, 40). Studies have started to reveal the role and importance of FMRP in patient and mouse models uncovering mRNA binding properties (5,29,17)

Within the past few years we have uncovered information linking FMRP to protein synthesis (30) and a micro-RNA dependent translational suppression pathway (23). In this review we will focus on explaining the neuronal functions and importance of FMRP in model systems. We will also propose a mechanism for both translational and transcriptional repression through a micro-RNA pathway.

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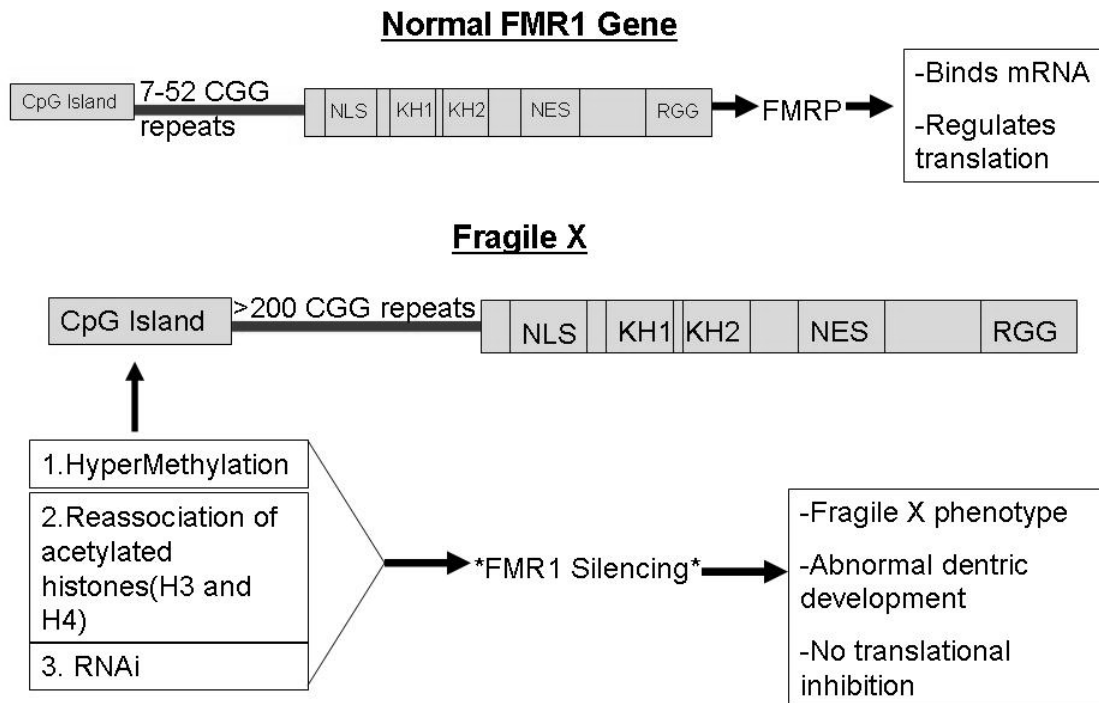


Figure 1. Molecular Basis of Fragile X Syndrome: A model of the normal FMR1 gene (top) and the fragile X FMR1 gene (bottom) with the defining difference in CCG repeat number between the two labeled. In the case of repeats >200 (fragile X syndrome), the CpG island is hypermethylated, histone 3 and 4 experience changes in acetylation, or RNA interference can all inhibit transcription of the FMR1 gene which codes for FMRP.

Functions of FMRP

In fetal tissue, FMRP is found to be most abundantly and universally expressed in the testis and the brain (21). The differentiation of neuronal stem cells related to the fragile X phenotype has shown alteration in fragile X patients linking the disease to development (10). FMRP was discovered to assert properties of RNA-binding proteins in areas of expression (40). The protein itself encompasses three RNA binding domains, two K domains and an RGG box (40, 15). Importantly, the FMRP protein maintains both a nuclear location signal (NLS) at the amino terminus end of the FMRP and a nuclear export signal (NES) encoded by exon 14 of the FMRP. These signals help to confirm RNA binding properties of FMRP by hinting at the possible translocation of various mRNAs via the FMRP pathway (17).

In order to bind mRNAs, FMRP forms an RNP complex containing FMRP, FRX1P, FRX2P, nucleolin, and three other proteins. The particles making up human RNPs are conserved in mice as well. (11). This RNP binds mRNA selectively by associating the G-quartet structure of the mRNA to the RGG box of the FMRP (5, 15). The G-quartet structure acts as a target for FMRP explaining the selective binding properties and the importance of transcriptional regulation via FMRP of the targeted mRNAs (15). FMRP binds directly to mRNAs via this interaction (9).

This direct interaction with mRNA allows FMRP to act as a translational control factor for many of these targeted mRNAs. Evidence for this was found because a large majority of FMRP is associated with ribosomes, translational machinery (38). Because the absence of the FMR1 gene was associated with fragile

X syndrome, a functional role of FMRP was investigated (38). FMRP was found to be associated with ribosomes in the dendritic structures of neurons elucidating a possible role of the proteins related to dendritic structure and neuronal plasticity (18). The association of FMRP with polyribosomes was eliminated in I304N mutations of extreme fragile X syndrome (19). The tissue specific expression of FMR1 connects the protein to important developmental structures in the brain such as the hippocampus (21). A set of FMR1 knockout mouse models were used to understand the relationship between dendritic formation and FMRP. Knock out mice were found to possess irregular dendritic spines (14). The importance of altered neuronal formation is discussed later.

The next important link connecting neuronal alterations and the function of FMRP is the evidence pointing to negative regulation of mRNA translation. By removing the binding site of FMRP we found a lack of translational inhibition (30, 31). Specifically, FMRP has been shown to regulate the production of MAP1B, a protein. In Futsch mutant mice show inverse regulation of MAP1B and altered synaptic growth (31, 45). This shows that FMRP possibly plays a major role in development of neuronal structure throughout the body.

mRNA ligands associated with FMRP

Because FMRP is shown to play significant roles in both mRNA transportation and translation, the characteristics of the specific mRNAs associated with FMRP have been explored. A variety of mRNAs are associated with FMRP. One of our recent studies examined 13 potential candidate mRNAs associated with FMRP and found that at least ten encode proteins

involved in synaptic plasticity or neuronal development. This data helped to identify some of the specific mRNA translation that is affected in mental retardation (15, 33). One specific mRNA was the mRNA coding for MAP1B. MAP1B is negatively regulated in the *Drosophila* nervous system. In the absence of FMRP, MAP1B experiences no inhibition and altered dendrite and axon development is noted (45).

We also interestingly found that FMRP associates with the non-translatable BC1 RNA. In addition to binding directly to FMRP, BC1 can associate with the FMRP target mRNAs in the absence of FMRP (43, 44, 45). This data suggests that BC1 is involved in specificity of FMRP to mRNAs and BC1 helps to inhibit translation of some mRNAs by blocking the initiation codon (43, 44). Large numbers of mRNAs have been recently associated with FMRP. Of these mRNAs, many have been found to differ in expression and distribution among wild type and FMR1 knockout mice (31). These recent studies identifying specific mRNAs associated with FMRP and their coded proteins have shown the importance of FMRP in neuronal development.

Altered neuronal development in Fragile X Syndrome

FMRP regulates the translation of many mRNAs which code for proteins involved in neuronal development. FMRP has been specifically found to be highly expressed in neurons. Areas controlling cognition are commonly affected in fragile X syndrome. A mouse model study shows deficient amygdala and hippocampal functions in FMR1 knockout mice during fear and conditioning tests (36). Specifically, FMRP is highly expressed in the dendritic formations of non-fragile X organisms (18). The dendritic functions encoded by the target mRNAs of FMRP are deficient in fragile X patients thus leading to altered synaptic function as observed in the fragile X phenotype (18).

Further proving the neuronal effects of FMR1 silencing mouse models have elucidated interesting findings in the area of altered synaptic plasticity related to fragile X mental retardation (22). Long term depression (LTD) dependent on glutamate receptors was found to be significantly altered in the hippocampus cells of knockout mice. By using DHPG to induce the glutamate dependent LTD, enhanced results were found in the knockout mice (22). This supports the earlier hypothesis that FMRP is important in regulating protein production in the synapse.

In mice lacking the expression of the FMR1 gene, irregular dendritic spines were observed (26) connecting the FMRP protein with synaptic growth. Dendritic spines in the visual cortex were compared among FMR1 knock out mice and wild type mice to explore the specific differences in dendritic growth. The dendrites of the knockout mice show a high incidence of long thin dendritic spines as well as dendritic spines of higher density (13). This data combined with the mRNAs targeted by FMRP that regulate neuronal structure connect fragile X syndrome with altered synaptic plasticity.

FMRP influences synaptic growth by normally inhibiting translation of functional mRNAs in synaptosomes (18). Two methods of the release of inhibition in normal models have been discovered: the first is dephosphorylation (12) and the second is excitation by glutamate receptors (3, 42). FMRP associated with stalled polyribosomes was consistently

phosphorylated where the FMRP associated with actively translating polyribosomes was consistently dephosphorylated in both brain cells and cultured cells (12). This is possibly the mechanism by which FMRP releases its inhibitory effect on targeted mRNAs.

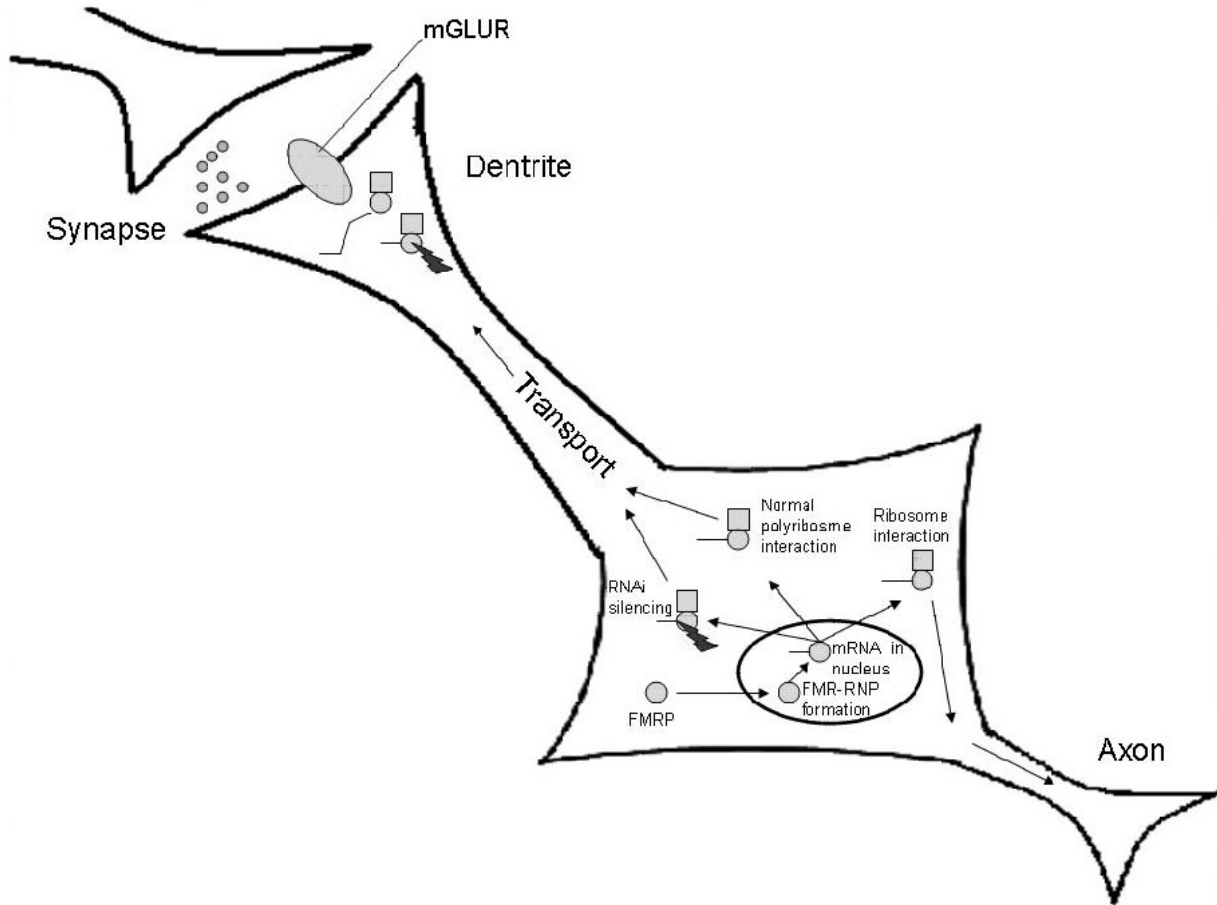
Recent models have associated the release of translational inhibition with the presence of mRNA granules and mGluR-induced translation (3). FMRP normally inhibits mRNA translation, but induction of translation has been associated with glutamate receptors. In fragile X syndrome mGluR-induced translation is heightened due to the lack of inhibition normally provided by FMRP. The decrease in mRNA granules in knockout mice supports this finding. Furthermore, upon excitation with an mGluR5 agonist increased granule levels were observed (3). The lack of rapid protein translation in sites mediated by neurotransmitters such as glutamate has been proposed to cause several abnormalities observed in fragile X syndrome (41).

The micro-RNA silencing of FMR1

The FMR1 gene is silenced by methylation induced by the CGG tri-nucleotide repeat expansion alone, but also by interaction with micro-RNAs (23, 24). FMRP interacts with Argonaute (AGO; 33) and components of the micro-RNA pathway such as Dicer (33, 24). In *Drosophila melanogaster* models the AGO ortholog was found to be suppressed in the presence of FMRP. When FMRP was removed the models experienced a loss of AGO suppression leading to a rough eye phenotype. Upon induced suppression of AGO, the rough eye phenotype was significantly reduced to levels of almost normal (24). AGO was found to be important to the biological functions of synapses, but not totally dependent on FMRP.

FMRP interacts with many molecules like AGO to influence translation. FMRP also has recently been associated with translational regulation through a micro-RNA pathway. Transcripts are produced from the expanded fragile X allele at some point early in development before complete methylation of the CpG island (23, 24, 34). These transcripts form structures referred to as hairpins that are cleaved by the enzyme Dicer (23) resulting in small mRNAs approximately 20 nucleotides in length. These small mRNAs communicate with the RITS complex, a transcriptional silencer of genes. The small RNAs direct the RITS complex to homologous mRNAs through complementary base pairing. The RITS complex recruits methylation machinery eventually leading to the suppression of the FMR1 gene as observed in fragile X syndrome (24). The RITS complex also may change the acetylation of specific histones (7, 24).

The FMR1 promoting region has shown to possess qualities of varying chromatin conformations linked to altered histones (20). When FMR1 is inactive as in fragile X cells the chromatin was displayed uniform conformation. In normal cells the interactions are far lesser than in fragile X cells (20). Histones acetylation is associated with normally expressed FMR1, but not the absence of FMR1 as in fragile X syndrome (7, 8, 20). Recent studies have treated fragile X cells with 5-aza-2-deoxycytidine and observed re-acetylation of histones 3 and 4. The re-acetylation of these histones resulted in FMR1 transcriptional reactivation (8). This finding presents an alley of treatment investigation for fragile X patients in the future.



Discussion

FMRP has shown tissue specific expression in areas of the brain and other parts of the body associated with the observed phenotype of fragile X syndrome such as the hippocampus and the testis. Specific binding of FMRP to various mRNAs has been elucidated also linking FMRP to an inhibitory role in the translation of respective mRNAs. Translation inhibition and mRNA targeting may be influenced by non-translatable mRNAs such as BC1. The silencing of the FMR1 gene is the central link in fragile X syndrome, however, recent studies have shown gene silencing by the binding of transcription factors to the promoter regions of FMR1 (28). This is data another example of the mechanism by which methylation silences the FMR1 gene.

The specific mRNAs associated with FMRP have proven to reveal a wealth of information explaining the gap of knowledge between the silencing of the FMR1 gene and the fragile X phenotype. MAP1B is an example of one such mRNA. MAP1B encodes for microtubule structural functions, thereby influencing physical features in the development of neurons. The absence of FMRP's regulatory influence leads to severely altered neurons in fragile X syndrome. Figure

2 proposes an overall view of the FMRP interactions in a normal neuron.

The alterations observed in fragile X syndrome are due to the loss of translational regulation via the various pathways that lead to transcriptional silencing of FMR1 (methylation of the CpG-island, micro-RNA interactions, and histone deacetylation). Altered synaptic plasticity leads to dysfunctional communication between neurons. This altered communication leads to the fragile X phenotype observed in humans.

The new insights into the AGO (in fly models, *Drosophila melanogaster*) and the micro-RNA pathway provide insight into a more specific cause of FMR1 silencing with possible drug treatment options. The mGluR induced translation provides the same exciting knowledge about translational regulation and drug treatment. Knowledge pertaining to further functions of FMRP still remains elusive along with the cause for the CGG tri-nucleotide repeat expansion which is thought to be the central cause behind fragile X syndrome.

Interesting studies have also shown the influence of environmental factors on fragile X phenotypes. When knockout mouse models were raised in enriched environments there were found to show increased dendrite branching, length, and dendrite spine density to levels near normal (39). This

provides non-invasive treatment options to counter the silencing of the fragile X gene, FMR1.

Transgenic genes have also been explored as a possible means of treatment. Because mouse models display some human-like symptoms in knockouts, a transgenic line of mice with yeast artificial chromosomes were generated. The study found behavioral and morphological changes. Also, over-expressing the gene did not have the opposite effect being an important consideration in treatment (37).

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References

1. Annemieke, J, M H. Verkerk, Maura Pieretti, James S. Sutcliffe, Ying-Hui Fu, Derek P. A Kuhl, Antonio P., Orly Reiner, Stephen Richards, Maureen F. Victoria, Fuping Zhang, Bert E. Eussen, Gert-Jan B. Van Ommen, Lau A J. Bloden, Gregory J. Riggins, Jane L. Chastain, Catherine B. Kunst, Jams Galjaard, Thomas Caskey, David L. Nelson, Ben Oostra, and Stephen T. Warren. "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 (1991): 905-914.
2. Anne, Vincent, Dominique Heitz, Christine Petit, Christine Kretz, Isabelle Oberle, and Jean-Louis Mandel. "Abnormal Pattern Detected in Fragile-X Patients by Pulsed-Field Gel Electrophoresis." Nature 349 (1991): 624-627.
3. Aschrafi, Armaz, Bruce A. Cunningham, Gerald M. Edelman, and Peter W. Vanderklish. "The Fragile X Mental Retardation Protein and Group I Metabotropic Glutamate Receptors Regulate Levels of the mRNA Granules in Brain." PNAS 102 (2005): 2180-2185.
4. Ashley, Claude T., James S. Sutcliffe, Catherine B. Kunst, Harold A. Leiner, Evan E. Eichler, David L. Nelson, and Stephen T. Warren. "Human and Murin FMR-1 Alternative Splicing and Translation Initiation Downstream of the CGG-Repeat." Nature Genetics 4 (1993): 244-250.
5. Ashley Jr., Claude T., Keith D. Wilkinson, Daniel Reines, Stephen T. Warren, and Stephen T. Warren. "FMR1 Protein: Conserved RNP Family Domains and Selective RNA Binding." Science 262 (1993): 563-565.
6. Bell, M V., M C. Hirst, Y Nakahori, R N. Mackinnon, A Roche, T J. Flint, P A. Jacobs, N Tommerup, L Tranebjærg, U Froster-Iskenius, B Kerr, G Turner, R H. Lindenbaum, R Winter, M Pembrey, S Thibodeau, and K E. Davies. "Physical Mapping Across the Fragile X: Hypermethylation and Clinical Expression of the Fragile X Syndrome." Cell 64 (1991): 861-866.
7. Bradford, Coffee, Fuping Zhang, Stephenie Ceman, Stephen T. Warren, and Daniel Reines. "Histone Modifications Depict and Aberrantly Heterochromatinized FMR1 Gene in Fragile X Syndrome." American Journal of Human Genetics 71 (2002): 923-932.

8. Bradford, Coffee, Fuping Zhang, Stephen T. Warren, and Daniel Reines. "Acetylated Histones are Associated with the FMR1 in Normal But Not Fragile X-Syndrome Cells." Nature Genetics 22 (1999): 98-101.
9. Brown, Victoria, Kersten Small, Lisa Lakkis, Yue Feng, Chris Gunter, Keith D. Wilkinson, and Stephen T. Warren. "Purified 10. Recombinant Fmrp Exhibits Selective RNA Binding as an Intrinsic Property of the Fragile X Mental Retardation Protein." The Journal of Biological Chemistry 273 (1998): 1521-15527.
10. Castren, Maija, Topi Tervonen, Virve Karkkainen, Seppo Heinonen, Ero Castren, Kim Larsson, Cathy E. Bakker, Ben Oostra, and Karl Akerman. "Altered Differentiation of Neuronal Stem Cells in Fragile X Syndrome." PNAS 102 (2005): 17834-17839.
11. Ceman, Stephanie, Victoria Brown, and Stephen T. Warren. "Isolation of an FMRP-Associated Messenger Ribonucleoprotein Particle and Identification of Nucleolin and the Fragile X-Related Proteins as Components of the Complex." Molecular and Cellular Biology 19 (1999): 7925-7932.
12. Ceman, Stephen, William T. O'donnell, Matt Reed, Stephana Patton, Jan Pohl, and Stephen T. Warren. "Phosphorylation Influences the Translation State of FMRP-Associated Polyribosomes." Human Molecular Genetics 12 (2003): 3295-3305.
13. Comery, Thomas A., Jennifer B. Harris, Patrick J Willems, Ben A. Oostra, Scott A. Irwin, Ivan Jeanne Weiler, and William T. Greenough. "Abnormal dendritic spines in fragile X knockout mice: Maturation and pruning deficits," PNAS 94 (1997): 5401-5404.
14. Syndrome in Fragile X Knockout Mice: Maturation and Pruning Deficits." Neurobiology 94 (1997): 5401-5404.
15. Darnell, Jennifer C., Kirk B. Johnson, Peng Jin, Victoria Brown, Stephen T. Warren, and Robert B. Darnell. "Fragile X Mental Retardation Protein Targets G Quartet MRNAs Important for Neuronal Function." Cell 107 (2001): 489-499.
16. The Dutch-Belgian Fragile. "Fmr1 Knockout Mice: a Model to Study Fragile X Mental Retardation." Cell 78 (1994): 23-33.
17. Eberhart, Derek E., Henry E. Malter, Yue Feng, and Stephen T. Warren. "The Fragile X Mental Retardation Protein is a Ribonucleoprotein Containing Both Nuclear Localization and Nuclear Export Signals." Human Molecular Genetics 5 (1996): 1083-1091.
18. Feng, Yue, Claire-Annd Gutekunst, Derek E. Eberhart, Hong Yi, Stephen T. Warren, and Steven M. Hersch. "Fragile S Mental Retardation Protein: Nucleocytoplasmic Shuttling and Association with Somatodendritic Ribosomes." The Journal of Neuroscience 17 (1997): 1539-1547.
19. Feng, Yue, Devin Absher, Derek E. Eberhart, Victoria Brown, Henry E. Malter, and Stephen T. Warren. "FMRP Associates with Polyribosomes as an MRNAP, and the I304N Mutant of Severe Fragile X Syndrome Abolishes This Association." Molecular Cell 1 (1997): 109-118.
20. Gheldof, Nele, Tomoko M. Tabuchi, and Job Dekker. "The Active FMR1 Promoter is Associated with a Large Domain of Altered Chromatin Conformation with Embedded Local Histone Modifications." PNAS 103 (2006): 12463-12468.
21. Hinds, Heather L., Claude T. Ashley, James S. Sutcliffe, David L. Nelson, Stephen T. Warren, David E. Housman, and Martin Schalling. "Tissue Specific Expression of FMR-1 Provides Evidence for a Functional Role in Fragile X Syndrome." Nature Genetics 3 (1993): 36-43.
22. Huber, Kimberly M., Sean M. Gallagher, Stephen T. Warren, and Mark F. Bear. "Altered Synaptic Plasticity in a Mouse Model of Fragile X Mental Retardation." PNAS 99 (2002): 7746-7750.

23. Jin, Peng, Daniela C. Zarnescu, Stephanie Ceman, Mike Nakamoto, Julie Mowrey, Thomas A. Jongens, David L. Nelson, Kevin Moses, and Stephen T. Warren. "Biochemical and Genetic Interaction Between the Fragile X Mental Retardation Protein and the MicroRNA Pathway." Nature Neuroscience 7 (2004): 113-117.
24. Jin, Peng, Reid S. Alisch, and Stephen T. Warren. "RNA and MicroRNAs in Fragile X Mental Retardation." Nature Cell Biology 6 (2004): 1048-1053.
25. Kenneson, Aileen, Fuping Zhang, Curt H. Hagedorn, and Stephen T. Warren. "Reduced FMRP and Increased FMR1 Transcription is Proportionally Associated with CGG Repeat Number in Intermediate-Length and Premutation Carriers." Human Molecular Genetics 10 (2001): 1449-1454.
26. Keokkeok, S K E, K Yamaguchi, B A Milojkovic, B R Dortland, T J H Ruijgrok, R Maex, W De Graaf, A E Smith, F Vanderwerf, C E Barker, R Willemsen, T Ikeda, S Kakizawa, K Onodera, D L Nelson, E Mientjes, M Joosten, E De Schutter, B A Oostra, M Ito, and C I De Zeeuw. "Deletion of FMR1 in Purkinje Cells Enhances Parallel Fiber LTD, Enlarges Spines, and Attenuates Cerebellar Eyelid Conditioning in Fragile X Syndrome." Neuron (47): 339-352.
27. Kremer, E J., M Pritchard, M Lynch, S Yu, K Holman, E Baker, S T. Warren, D Schlessinger, G R. Sutherland, and R I. Richards. "Mapping of DNA Instability At the Fragile X to a Trinucleotide Repeat Sequence P(CGG)N." Science 252 (1991): 1711-1714.
28. Kumari, Daman, and Karen Usdin. "Interaction of the Transcription Factors USF1 USF2, and a-Pal/Nrf-1 with the 28.
29. Kunst, Catherine B., and Stephen T. Warren. "Cryptic and Polar Variation of the Fragile X Repeat Could Result in Predisposing Normal Alleles." Cell 77 (1994): 853-861.
30. Li, Zhenzhong, Youyi Zhang, Keith D. Wilkinson, Stephen T. Warren, and Yue Feng. "The Fragile X Mental Retardation Protein Inhibits Translation Via Interacting with MRNA." Nucleic Acids Research 29 (2001): 2276-2283.
31. Lu, Robert, Houping Wang, Zhe Liang, Li Ku, William T. O'donnell, Wen Li, Stephen T. Warren, and Yue Feng. "The Fragile X Protein Controls Microtubule-Associated Protein 1B Translation and Microtubule Stability in Brain Neuron Development." PNAS 101 (2004): 15201-15206.
32. Malter, Henery E., Jane C. Iber, R Willemsen, Esther D. Graaff, Jack C. Tarleton, J Leisti, Stephen T. Warren, and Ben A. Oostra. "Characterization of the Full Fragile X Syndrome Mutation in Fetal Gametes." Nature Genetics 15 (1997): 165-169.
33. Miyashiro, Kevin Y., Andrea Beckel-Mitchener, T Patrick Purk, Kevin G. Becker, Tanya Barret, Lei Liu, Salvatore Carbonetto, Ivan Jeanne Weiler, William T. Greenough, James Eberwine, and James Eberwine. "RNA Cargoes Associating with FMRP Reveal Deficits in Cellular Functioning in Fmr1 Null Mice." Neuron 37 (2003): 417-431.
34. Novina, Carl D., and Phillip A. Sharp. "The RNAi Revolution." Nature 430 (2004): 161-164.
35. Oberle, I, F Rousseau, D Heitz, C Kretz, D Devys, A Hanauer, J Boue, M F. Bertheas, and J L. Mandel. "Instability of a 550-Base Pair DNA Segment and Abnormal Methylation in Fragile X Syndrome." Science 252 (1991): 1097-1102.
36. Paradee, W, H E. Melikian, D L. Rasmussen, A Kenneson, P J. Conn, and S T. Warren. "The Fragile X Mouse: Stain Effects of Knockout Phenotype and Evidence Suggesting Deficient Amygdala Function." Neuroscience 94 (1999): 185-192.
37. Peier, Andrea M., Kellie L. McIlwain, Aileen Kenneson, Stephen T. Warren, Richard Paylor, Richard Paylor, and David L. Nelson. "(Over)Correction of FMR1deficiency with YAC Transgenics: Behavioral and Physical Features." Human Molecular Genetics 9 (2000): 1145-1159.
38. Pieretti, Maura, Fuping Zhang, Ying-Hui Fu, Stephen T. Warren, Ben A. Oostra, C Thomas, and David L. Nelson. "Absence of Expression of the FMR-1 Gene in Fragile X Syndrome." Cell 66 (1991): 817-822.
39. Restivo, Leonardo, Francesca Ferrai, Enrica Passino, Carmelo Sgobio, Jorg Bock, Ben Oostra, Claudia Bagni, and Martine Ammassari-Teule. "Enriched Environment Promots Behavioral and Morphological Recovery in a Mouse Model for the Fragile X Syndrome." PNAS 102 (2005): 11557-11562.
40. Siomi, Haruhiko, Mikiko C. Siomi, Robert L. Nussbaum, and Gideon Dreyfuss. "The Protein Product of Fragile X Gene, FMR1, Has Characteristics of an RNA-Binding Protein." Cell 74 (1993): 291-298.
41. Sutcliffe, James S., David L. Nelson, Fuping Zhang, Maura Pieretti, C Thomas, Debra Saxe, and Stephen T. Warren. "DNA Methylation Represses FMR-1 Transcription in Fragile X Syndrome." Human Molecular Genetics 1 (1992): 397-400.
42. Christine K. Base, and William T. Greenough. "Fragile X Mental Retardation Protein is Necessary for Neurotransmitter-Activated Protein Translation At Synapses." PNAS 101 (2004): 17504-17509.
43. Zalfa, Francesa, Marcello Glorgi, Beatrice Primerano, Annamaria Moro, Alessandra Di Penta, Surya Reis, Ben Oostra, and Claudia Bagni. "The Fragile X Syndrome Protein FMRP Associates with BC1 RNA and Regulates the Translation of Specific MRNAs At Synapses." Cell 112 (2003): 317-327.
44. Zalfa, Francesa, Marcello Giogri, Beatrice Primerano, Annamaria Moro, Alessandra Di Penta, Surya Reis, Ben Oostra, and Claudia Bagni. "The Fragile X Syndrome Protein FMRP Associates with BC1 RNA and Regulates the Translation of Specific MRNAs At Synapses." Cell 112 (2003): 317-327.
45. Q. Hang, Yong, Adina M. Bailey, Henreich J G Matthies, Robert B. Renden, Mark A. Smith, Sean D. Speese, Gerald M. Rubin, and Kendal Broadie. "Drosophila Fragile X-Related Gene Regulates the MAP1B Homolog Futsch Control Synaptic Structure and Function." Cell 107 (107): 591-60.